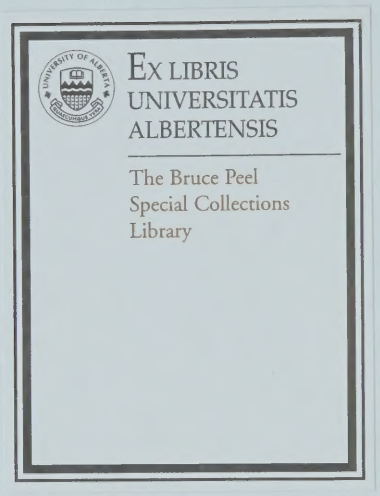
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"It is the mark of an educated mind to be able to entertain a thought without accepting it."

Aristotle **(384 BC - 322 BC)**

UNIVERSITY OF ALBERTA

NEUROCIRCUITRY OF SOCIAL PHOBIA: FUNCTIONAL MAGNETIC RESONANCE IMAGING (fMRI) OF A PUBLIC SPEAKING TASK

BY

EMILY C. BELL

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitiled Neurocircuitry of social phobia: functional magnetic resonance imaging (fMRI) of a public speaking task by EMILY CLAIRE BELL in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

DEDICATION

To my parents, whose commitment to lifelong learning has provided me with boundless opportunities to expand my knowledge and my education. For their constant love, support and encouragement, without which this would never have been possible.

ABSTRACT

Neurocircuitry of Social Phobia: functional Magnetic Resonance Imaging (fMRI) of a public speaking task.

OBJECTIVE: Investigate the neurocircuitry involved in the fear of public speaking, in patients with social phobia, using fMRI. **BACKGROUND**: Social phobia (SP) is a disorder characterized by a chronic anxiety of social or performance situations, often including an intense fear of public speaking. **METHODS**: Fourteen subjects suffering from generalized SP and fourteen healthy control subjects underwent fMRI. Functional images were acquired during performance of speaking tasks, in private and public. Preprocessing steps and statistical analysis were carried out using Statistical Parametric Mapping 99 (SPM99). **RESULTS**: Patients with SP exhibited increased activation of the precentral gyrus, the cerebellum and temporal lobe gyri during private and public speech, compared to baseline non-speaking conditions. In the same analysis, increased activation in controls was observed primarily in the precentral gyrus. **CONCLUSION**: SP patients show increased brain activity during private and public speaking, relative to baseline, in brain areas involved in fear and anxiety.

PREFACE

Despite recent progress achieved by neuroimaging studies, our understanding of the pathophysiological mechanisms and neurocircuitry involved in the anxiety associated with social phobia (SP) remains largely uncertain. Functional neuroimaging studies investigating psychiatric disorders have recently focused on paradigms employing symptom provocation. This has resulted in a greater insight into the possible neuroanatomical correlates of anxiety in many simple phobias, in post-traumatic stress disorder (PTSD) and in SP (Pissiota et al., 2002; Bremner et al., 1999; Shin et al., 1999 Fredrickson et al., 1997; Rauch et al., 1995). Recent progress in functional neuroimaging has encouraged the development of disorder-specific symptom provocation paradigms in the investigation of psychiatric disorders.

Fear of public speaking is common and prevalent. In the late 1990's, a Canadian community survey of approximately 500 individuals investigated the prevalence of fear of public speaking. In this sample, 34% reported that they suffered from an excessive fear of speaking to large audiences (Stein et al., 1996b). Moreover, within the population of individuals describing a significant fear of public speaking, 49 out of the 167 individuals reported that their speaking anxiety had resulted in sufficient disability or distress to allow fulfilment of DSM-IV criteria for SP (Stein et al., 1996b).

As development of our current study began, a group of researchers led by Maria Tillfors published the results of their investigation of cerebral blood flow during a stressful speaking task in patients with SP (Tillfors et al. 2001). We were greatly

interested by the use of an authentic private and public speaking paradigm in this positron emission tomography (PET) study to assess changes in regional cerebral blood flow (rCBF). Prior to the publication of the Tillfors et al. study, we had subjected several patients to fMRI scans, employing an auditory autobiographical script paradigm. For each patient this script reflected certain features of their personal experience with anxiety in public speaking. We predicted that the scripts would successfully provoke anxiety symptoms in these patients. Unfortunately, we did not observe high subjective ratings of anxiety in the patients performing this task, nor did they report that they had experienced the normal symptoms or normal magnitude of symptoms associated with their past efforts in public speaking.

With the knowledge that fear of public speaking was common among patients with SP, and having observed the successful employement of a public speaking task by Tillfors et al. (2001), we initiated our study investigating the neurocircuitry of SP using an actual public speaking paradigm designed to provoke and replicate the social anxiety experienced by these individuals. Our study is the first of its kind to assess the functional neuroanatomy of SP using fMRI, a tool which provides greater spatial and temporal resolution than PET. Furthermore, it is a study using a unique paradigm design, one which challenges limitations considered to be present in fMRI study design (e.g. the assumption that the performance of an overt speech task during imaging would result in significant head motion and induce signal artifacts). This thesis will explore what is understood to date regarding the prevalence and comorbidities of SP, the neurobiological mechanisms involved in SP, and the neuroanatomy of anxiety and fear. Furthermore,

what follows will address the principles of nuclear magnetic resonance (NMR) imaging, the principles of fMRI, and the relationship between functional neuroimaging and brain activity. These topics will converge at, and contribute to, an understanding of the study we have conducted, an investigation of the neurocircuitry of social anxiety during public speaking.

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LIST OF ABBREVIATIONS

α	alpha
β	beta
[^{123}I] β-CIT	[^{123}I] 2β-carbomethoxy-3β-(4-iodophenyl)tropane
[^{123}I] IBZM	[^{123}I] iodobenzamide
BDI	Beck Depression Inventory
BOLD	Blood oxygen level-dependent
CBT	Cognitive behavioural therapy
Cl^-	Chloride ion
CS+	Conditioned stimulus
DSM-IIIR	Diagnostic and Statistical Manual of Mental Disorders, Third edition revised
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, Fourth edition
EPI	Echo planar imaging
fMRI	Functional magnetic resonance imaging
FP	Follicular phase
GABA	Gamma-aminobutyric acid
GH	Growth hormone
GSP	Generalized social phobia
HAM-A	Hamilton Anxiety Scale
HC	Healthy control
HVA	Homovanillic acid

K^+	Potassium ion
LSAS	Liebowitz Social Anxiety Scale
LTP	Long term potentiation
*m*CPP	*meta*-chlorophenylpiperazine
MDD	Major Depressive Disorder
min	minute
MRI	Magnetic Resonance Imaging
MRS	Magnetic resonance spectroscopy
MT	Middle temporal
Na^+	Sodium ion
NAS	Neuroactive steroid
NCS	National Comorbidity Survey
NMR	Nuclear magnetic resonance
PET	Positron emission tomography
PFC	Pre-frontal cortex
PRCS	Personal Report of Confidence as a Speaker
PREGS	Pregnenolone sulfate
PTSD	Post-traumatic Stress Disorder
rCBF	Regional cerebral blood flow
RF	Radio-frequency
s	second
SCID	Structured Clinical Interview for DSM-IV
SP	Social Phobia

SPECT	Single photon-emission computed tomography
SPM99	Statistical Parametric Mapping 99
SSP	Specific social phobia
SSRI	Selective serotonin reuptake inhibitor
US-	Unconditioned stimulus
VAS	Visual analogue scale
V1	Primary visual cortex
V5	Visual association cortex

A. INTRODUCTION

CHAPTER 1

Social Phobia: Background

1.1 Definition, prevalence, comorbidity

Social phobia (SP), or social anxiety disorder, is a pervasive and chronic psychiatric disorder which causes significant daily functioning impairment for sufferers. With a reported lifetime prevalence as high as 13.3%, and a 12-month prevalence rate of 7.9%, as reported by the National Comorbidity Survey (NCS) conducted in the mid-1990's (Kessler et al., 1998), SP ranks as the most common anxiety disorder in the United States (Fones et al., 1998). Of disorders classified as mental illness, only major depressive disorder (MDD) and alcohol dependence have a higher prevalence rate than SP (Fones et al., 1998; Ballenger, 2000).

The principal diagnostic feature of SP, according to the Diagnostic Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association), is a "marked and persistent fear of one or more social or performance situations" accompanied by a fear of scrutiny or judgement by others (Ballenger, 2000). Furthermore, patients fear that, in social situations, they may be humiliated or may show embarrassing signs of anxiety (Ballenger, 2000). The anxiety experienced is often accompanied by the appearance of somatic symptoms such as heart palpitations, trembling, sweating, dry mouth, hot or cold flashes, and headache (Fones et al., 1998). Consequently, individuals suffering from SP often avoid a variety of social situations such as speaking in public, using a telephone, eating in public, attending parties, urinating in a public washroom and

meeting new people (Fones et al., 1998). Furthermore, for a diagnosis of SP, the sufferer must recognize that the anxiety experienced in social situations stems from an unreasonable or excessive fear (Fones et al., 1998). In individuals suffering from SP, exposure to feared social or performance situations consistently and immediately evokes anxiety and distress, which may be subdued by the avoidance of the same (Fones et al., 1998). The anxiety (or avoidance) of social settings experienced by patients with SP results in significant daily functioning impairment, interfering with the patient's normal routine, occupational functioning, academic functioning or interaction in social relationships (Ballenger, 2000); this is a key feature in making a diagnosis of SP (Moutier and Stein, 1999).

SP generally begins early in life, in childhood or adolescence, with a mean age at onset between 13 and 20 years (Davidson et al., 1993a; Ballenger, 2000 and Moutier and Stein, 1999; Chartier et al., 1998). Indeed, findings from the Duke Epidemiological Catchment Study (1993a) have suggested that onset of SP after age 25 is unusual (Moutier et al., 1999). Across genders, SP occurs more often in women (female-to-male ratio of 3 to 2) (Moutier and Stein, 1999), although men are more likely to seek treatment (Weinstock, 1999). Sociodemographic studies indicate that patients with SP are less likely to marry, more likely to divorce and more likely to live in a low socio-economic bracket and have a low level of education (Schneier et al., 1992).

There is a high incidence of comorbidity with other psychiatric disorders in patients with SP. In fact, comorbid psychiatric disorders are absent in only about one-

third of patients with SP (Schneier et al., 1992), and most often the social anxiety is present in advance of the onset of any comorbidity (Moutier and Stein, 1999). Results from the National Comorbidity Survey (1993) indicate that individuals with SP are 4 times more likely to develop depression and 9 times more likely to develop panic disorder or generalized anxiety disorder than individuals not suffering from SP, over their lifetime. Furthermore, social phobics are 10.4 times more likely to develop agoraphobia than non-sufferers (Kessler et al., 1994). Moreover, patients suffering from SP are at greater lifetime risk for the development of simple phobia, obsessive compulsive disorder, alcohol abuse and drug abuse (Reiger et al., 1990; Kessler et al., 1998; Schneier et al., 1992; Moutier and Stein, 1999). Studies have reported some comorbidity with SP in patients suffering from bipolar disorder and schizophrenia (Cosoff et al., 1998; Colom et al., 2001), and a relatively high prevalence of SP has been observed in patients suffering from anorexia nervosa and bulimia (16 out of 29 patients and 20 out of 34 patients; respectively) (Godart et al., 2000). Additionally, an exceptionally high comorbidity rate exists between GSP and avoidant personality disorder. With comorbidity rates ranging in reports from 22%-89% between these two disorders, most researchers suggest that avoidant personality disorder represents merely a more severe form of GSP, rather than a distinct disorder (Chavira and Stein, 2002).

The presence of comorbid disorders in patients suffering from SP can greatly impact the course and severity of the disease (Erwin et al., 2002). For example, the lifetime suicide attempt rate for individuals suffering from SP without comorbidity is 1.0%; this is in contrast to by a much higher rate of 15.7% among patients who suffer

from SP accompanied by comorbid disorder(s) (Schneier et al., 1992). Furthermore, a recent study conducted by Erwin and collegues (2002) has assessed social phobic symptoms in patients with pure SP, patients with SP and a comorbid anxiety disorder, and patients with SP and a comorbid mood disorder. An assessment of symptom severity revealed that patients in the SP comorbid mood disorder group suffered from significantly more severe social anxiety disorder symptoms than patients with pure SP (Erwin et al., 2002). Patients suffering from SP with an additional anxiety disorder did not show significantly different symptom severity from patients with pure SP (Erwin et al., 2002).

Further research has explored a possible relationship between social anxiety and alcohol dependence and abuse. In their research on alcoholics, Schuckit et al. (1997) found an increased lifetime prevalence rate of 3.2% of social anxiety disorder, which almost doubled the prevalence they found in nonalcoholics. With the intention of explaining the increased incidence of substance abuse in SP patients, it has been suggested that patients use alcohol "self-medication" as a technique to reduce the anxiety experienced in social settings (Schneier et al., 1992). However, a recent study by Crum and Pratt (2001) evaluating the risk of alcohol use disorders in SP contradicts this notion. Analysis revealed that a diagnosis of SP, at the time of follow-up, was not associated with an increased risk for heavy drinking or alcohol abuse. However, patients who were diagnosed with subclinical SP (patients who do not avoid the social situations) did demonstrate high risks of heavy drinking, and a high risk of alcohol abuse or dependence, at follow-up (Crum and Pratt, 2001). The authors suggest that individuals suffering from

subclinical SP, as they do not avoid social situations, are at greater risk for alcohol use disorders because these individuals may use alcohol as a means of decreasing anxiety in social situations. However, patients with diagnosed SP are likely not at a similar risk for developing alcohol-related problems, as they are able to reduce their social anxiety by avoiding social situations altogether (Crum and Pratt, 2001).

Davidson et al. (1993) reported, in their investigation of a group of social phobics, a mean duration of illness of 19.4 years. More recently, however, a study by Chartier et al. (1998) reporting on a nonclinical community sample of individuals with SP has outlined the course of the illness. In their group of subjects (39 patients meeting the criteria for lifetime occurrence of SP), Chartier et al. (1998) reported a mean duration of illness of 29.0 years, almost ten years greater than that reported by Davidson and colleagues (1993). Moreover, based on the interview 3 patients were classified as having an illness which worsened over its course, 13 were classified to have unchanged severity of the illness over time and 8 patients were "improved" over time. In the sample, 15 patients no longer met the criteria for SP at the time of the interview, and were classified to be in remission (Chartier et al., 1998). In their extensive study of the natural course of SP, Chartier and collegues (1998) also investigated factors impacting the course of the illness. At interview, the patient responses suggested that numerous factors e.g. family environment, self-esteem, poverty, comorbidity with other disorders, maturity, support from others (respected individuals) and life events, influenced the onset and natural course of the illness.

It is generally acknowledged that two distinct subtypes of SP exist, generalized social phobia (GSP) and specific social phobia (SSP) (Liebowitz, 1999). Patients suffering from the generalized form of the disorder experience anxiety in most social situations, in public performance settings and in less structured social interaction settings (Moutier and Stein, 1999). This is in contrast to patients suffering from the nongeneralized or specific disorder (SSP), also called discrete SP, who experience anxiety in only one or two social situations, while remaining relatively comfortable in others (Fones et al., 1998; Moutier and Stein, 1999). Generally, in SSP patients, the anxiety experienced occurs in performance situations, such as public speaking, performing in front of a group, writing while being observed or eating in front of others (Moutier and Stein, 1999).

Studies investigating the two subtypes of social anxiety disorder have concluded that patients with the generalized form of the disorder suffer greater impairment, greater anxiety and depression, and increased comorbidity than patients with the nongeneralized subtype (Brunello et al., 2000; Kessler et al., 1998). Earlier, Heimberg and collegues (1990) investigated the differences between 35 patients with DSM-IIIR classified GSP and 22 social phobic patients suffering from only a fear of public speaking (SSP). Between these two groups, the analysis of demographic data revealed the GSP patients were younger, less educated and less likely to be employed than those with a restricted fear of public speaking (Heimberg et al., 1990). Furthermore, this study found that GSP patients were more severely impaired than the SSP patients and that GSP patients reported greater social avoidance and distress and greater fears of negative evaluation

compared to public speaking phobics (Heimberg et al., 1990). In contrast, Weinshenker et al. (1996/1997) have reported, in their study of 176 patients with SP, no statistically significant difference in comorbidity at the time of the study, in age of onset or in quality of life measures (health, and social and emotional functioning) between SSP patients and GSP patients. The authors did, however, observe a trend towards a lower level of functioning, by analysis of global assessment score, in patients with GSP compared to patients with SSP.

Of interest, when exposed to a behavioral challenge, these two samples show distinct patterns of heart rate acceleration. Exposure to a behavioral challenge (initiating conversation, public speaking) resulted in increased heart rate reactivity (heart rate at each time point minus baseline) in the specific public speaking phobia group compared to the GSP patients. Greater heart rate reactivity in comparison to GSP patients, in fact, was observed in the SSP patients throughout the entire task. Furthermore, the SSP patients experienced a significant decrease in heart rate reactivity during the challenge after an initial peak, while heart rate in GSP patients remained relatively stable over the course of the challenge (Heimberg et al., 1990). Heimberg et al.'s (1990) observation of increased heart rate reactivity in SSP patients, in response to anxiety provocation challenge, is supported by numerous studies (Boone et al., 1999; Levin et al., 1993). The authors maintain that this difference in cardiovascular response clearly distinguishes the SSP patients from those with GSP (Heimberg et al., 1990; Boone et al., 1999).

The delineation between the two subtypes of social anxiety is still, to date, unclear. Among most researchers, it is widely maintained that GSP patients suffer greater impairment and greater rates of comorbidity than those with a nongeneralized form of the disorder (Kessler et al., 1998; Fones et al., 1998). Analysis of the genetic influence on the development of SP also suggests that the two subtypes are relatively distinct. Stein et al. (1998) have reported a 26.4% incidence of GSP among first-degree relatives of individuals with GSP. This is in contrast to the 2.7% incidence they observed in subjects without SP. Moreover, there was no significant difference in the incidence of SSP in the first-degree relatives of patients with GSP or the individuals without SP (Stein et al., 1998). The authors maintain that if the two subtypes could merely be differentiated on the basis of severity then the incidence of SSP would also have been higher in the relative group of patients with GSP (Stein et al., 1998). The results of this study support the delineation between the GSP and SSP subtypes of the disorder. On the other hand, however, the suggestion that these subtypes are aligned on a continuum, rather than two distinct entities, remains under investigation (Weinshenker et al., 1996/1997).

Researchers have questioned whether hereditary factors might play a role in the development of SP. Previous studies have reported that first-degree relatives of patients with the generalized form of the disorder are at greater risk for the development of SP compared to the first-degree relatives of control subjects (Stein et al., 1998; Fyer et al., 1993; Reich et al., 1988). Twin studies conducted by Stein et al. (2002b) and Sundet et al. (2003) have investigated the heritability of social fears among monozygotic and dizygotic twins. The former study, investigating the fear of negative evaluation [a

prevalent fear among patients with SP (Turner et al., 1992)] among 437 twin pairs, observed a higher intrapair correlation in monozygotic twins (male and female pairs), compared to dizygotic twins (male and female pairs). This difference in correlation between monozygotic (genetically identical) and dizygotic (nonidentical) twins suggests that genetic factors play some role in the fear of negative evaluation. Moreover, Stein et al. (2002b) concluded that additive genetic influences were responsible for about 42 % of the variation in the fear of negative evaluation responses observed, and that non-shared environmental factors accounted for another approximately 58% of this variation. A recent investigation of social fears (including fears of being criticized, meeting people in positions of authority, looking silly, being self-conscious and being misunderstood) has reported a genetic heritability component of approximately 22% and a environmental effect of approximately 78% on self-reported evaluation of social fears in a sample of monozygotic twins and their families (Sundet et al., 2003).

Furthermore, in the investigation of factors influencing the course of illness in SP, researchers have explored the association between behavioral inhibition and social anxiety. Children suffering from behavioral inhibition, who display increased fear and shyness in unfamiliar situations, also experience an increased rate of social anxiety disorder (Beiderman et al., 2001; Mick et al., 1998). This result can be startling; in the child group assessed by Biederman and colleagues (2001), there was a 17% incidence of SP or avoidant personality disorder in the children with behavioral inhibition, contrasted by a 5% incidence in children not displaying behavioral inhibition. Moreover, behavioral inhibition does not predict a higher occurrence of generalized anxiety disorder in these

individuals (Mick et al., 1998) and is independent of parental psychiatric diagnosis (Bierderman et al., 2001). The evidence in this area suggests that the childhood presence of behavioral inhibition could be a factor specifically influencing or predictive of future adult social anxiety.

1.2 Neurobiology

To date, the biological basis of social anxiety disorder has not been comprehensively investigated and, while many studies have considered the involvement of various neurotransmitter systems in this disorder, our understanding of biological dysfunction in SP is still evolving. The research underlying a dysregulation of neurotransmitter systems in SP is compiled in this section.

Serotonin:

The efficacy of selective serotonin reuptake inhibitors (SSRIs), considered first-line pharmacotherapy in treating the anxiety experienced by SP patients, supports the notion of serotonergic dysfunction in these individuals. Moreover, observations by Knutson et al. (1998), in healthy controls, that social affiliation (cooperative behavior during a puzzle task) can be increased with the administration of an SSRI, suggests that serotonin plays a role in social behavior. Investigations of serotonergic function in SP by means of pharmacological challenge have been applied in humans. However, the degree to which an abnormal hormonal response can be applied to reflect a more generalized central neurotransmitter dysfunction is unclear (Agryropoulos et al., 2001). In early studies conducted by Tancer and collegues (1994/1995), cortisol and prolactin responses

to fenfluramine administration were measured in GSP patients. Pharmacological challenge with fenfluramine, which causes increased release of serotonin at the synapse, has previously been used to characterize central serotonergic function in various psychiatric disorders (Siever et al., 1984; Targum and Marshall, 1989; Hollander et al., 1998). After administration of fenfluramine, an increased cortisol response was observed in social phobics compared to healthy controls. In contrast, no difference in prolactin response between SP patients and healthy controls was observed (Tancer et al., 1994/1995). These results are comparable to those observed by Hollander et al. (1998) after administration of *meta*-chlorophenylpiperazine (*m*CPP), a serotonin agonist, in which patients with SP demonstrated an increased cortisol response, but unchanged prolactin response compared to controls. Upon analysis of their results, Tancer and collegues (1994/1995) postulated that the abnormal response to fenfluramine observed in SP patients might reflect a serotonergic receptor hypersensitivity, possibly at specific serotonergic receptors responsible for cortisol release, not prolactin release.

Additional evidence for the influence of the serotonin system in social behavior and social anxiety comes from observations made in primate models of dominance and subordination. Observations that primate subordinates spend an increased amount of time alone and an increased amount of time scanning their environment compared to dominant primates has lead researchers to investigate the biological correlates in these animals, both dominant and subordinate, in an attempt to better understand SP within the context of an animal model (Shively 1998; Mathew et al., 2001). It would seem that increasing serotonergic function (by means of SSRI administration) in primates is

associated with an increase in dominant behavior and an increase in social interaction (Raleigh et al., 1991). In contrast to results obtained in humans suffering from SP, an investigation by Botchin et al. (2001) of laboratory-housed monkeys revealed a blunted prolactin release to the fenfluramine challenge. This altered response which is indicative of serotonergic dysfunction, was observed in primates who were socially withdrawn and spent less time in passive body contact than non-laboratory housed primates. Moreover, monkeys who reacted with a high prolactin response demonstrated less social withdrawal (Botchin et al., 2001). It would seem that in both primate models of social anxiety and patients suffering from SP, serotonin function is dysregulated, causing abnormal response to serotonergic pharmacological probes.

Dopamine:

The possibility of dopaminergic dysfuntion in SP has been previously suggested by case study reports and by clinical evidence observed in patients suffering from disorders characterized by dopaminergic dysfunction (Agryropoulos et al., 2001). A case study of 15 patients suffering from Tourette's disorder and treated with low-dose haloperidol, a dopamine receptor blocker, reported the emergence of school or work avoidance and symptoms consistent with a social anxiety disorder diagnosis (Mikkelson et al., 1981). These symptoms, which appeared within a few weeks of treatment with haloperidol, were eradicated by discontinuation or reduction of the medication (Mikkelson et al., 1981). Furthermore, a high rate of SP has been reported in individuals suffering from Parkinson's disease, a degenerative disease resulting in destruction of

dopaminergic neurons in the striatum (Agryropoulos et al., 2001). For many patients, SP was present prior to the emergence of Parkinson's disease (Agryropoulos et al., 2001).

The investigation of central dopaminergic dysregulation in SP has also been assessed by pharmacological challenge. Tancer and collegues (1994/1995) tested 21 GSP patients for prolactin suppression and increased spontaneous eye-blink rate response to levodopa, a dopamine agonist. These responses to levodopa have been used as measures of dopamine function in the tuberoinfudibular and nigrostriatal systems (Tancer et al., 1994/1995). Compared to healthy controls, there was no significantly greater prolactin suppression in patients suffering from SP in response to levodopa administration. Moreover, spontaneous eye-blink response to levodopa was not significantly different between the two groups (Tancer et al., 1994/1995). The authors suggest that the lack of overt evidence of dopamine dysfunction in SP patients in their study, however, may be due to other confounding effects (floor effect in prolactin suppression, problems stemming from the pharmacaological probe levodopa) (Tancer et al., 1994/1995). In fact, this thesis will later discuss dysregulations of the brain dopamine system reported in neuroimaging investigations of subjects with social anxiety disorder.

Noradrenaline:

Noradrenaline is fundamentally involved in the regulation of the autonomic nervous system. Theories of noradrenergic abnormalities in SP have arisen mainly due to its involvement in the regulation of autonomic response and observations that this system is hyperaroused (resulting in symptoms such as blushing, heart palpitations, sweating) in performance or social situations in individuals suffering from social anxiety (Mathew et

al., 2001; Agryropoulos et al., 2001). Central noradrenergic activity, assessed by growth hormone (GH) response to the alpha$_2$-adrenergic receptor agonist clonidine has been assessed and compared in patients suffering from GSP, patients with panic disorder and in healthy controls. In the study by Tancer et al. (1993), a similarly blunted GH response to clonidine was observed in patients diagnosed with SP and patients with panic disorder, compared to healthy controls. However the same challenge in GSP patients revealed no statistically significant abnormality in GH response to oral clonidine administration, when compared to controls (Tancer et al., 1994/1995). Varied reports of plasma noradrenaline levels at baseline and during stressful situations have been described, some indicating abnormally high levels of noradrenaline in social phobics, others reporting no abnormalities (Agryropoulos et al., 2001; Tancer et al., 1994/1995; Stein 1992). Additionally, increasing plasma epinephrine levels by epinephrine influsions in individuals suffering from SP is insufficient to produce social anxiety (Papp et al., 1988).

Gamma-Aminobutyric Acid (GABA):

The success of benzodiazepines, which modulate GABA receptor activity, coupled with the efficacy of medications such as the anticonvulsant gabapentin, a GABA analogue (Jefferson, 2001), in the treatment of SP suggest that the GABAergic system may play a role in this disorder. Moreover, alterations have been observed in baseline levels of pregnenolone sulphate (PREGS), a steroid having agonist and antagonist properties (depending on the concentration) at the $GABA_A$ receptor, in patients suffering from GSP (Heydari and LeMelledo, 2002). Although not yet analyzed, I have collected blood samples at baseline and following the Social Trier Test (which includes a public

speaking task) to assess neuroactive steroid (NAS) levels. These data should allow in depth analysis in SP patients of the levels of these steroids, which act on the $GABA_A$ receptor. This, we hope, will contribute to a greater understanding of the magnitude of the involvement of the $GABA_A$ receptor, and NAS dysregulations, in the pathophysiology of SP.

CHAPTER 2

Neuroimaging Techniques: BOLD fMRI

2.1 Neuroimaging techiniques: PET, SPECT, MRS

While the research and emphasis in this thesis will focus on using functional magnetic resonance imaging (fMRI) in the determination of the psychopathology of SP, there are numerous other neuroimaging techniques which have provided insight into the neurocircuitry behind a spectrum of psychiatric disorders, including SP. A description of neuroimaging techniques (positron emission tomography, single photon emission tomography and magentic resonance imaging) as well as an in-depth overview of fMRI, are included in this chapter.

Positron emission tomography (PET):

Neuroimaging using PET technology allows the assessment of many different metabolic processes occurring in the brain. For example, PET allows the determination, in humans, of regional cerebral blood flow, receptor density, receptor binding potential, and drug activity *in vivo*. By radiolabelling molecules with positron-emitting isotopes of carbon, nitrogen, oxygen or fluorine (accomplished by injecting a radioactive tracer into a peripheral vein), the position and concentration of the molecules involved in the specific metabolic activity under investigation can be traced by following the emission of gamma rays in the PET scanner (Berger, 2003;Gordon, 1999).

Single photon emission computed tomography (SPECT):

In contrast to PET, SPECT is a technique which uses injection of gamma-emitting isotopes, rather than positron-emitting isotopes, to radiolabel and trace molecules in the brain. These radiolabelled isotopes are specifically selected based on their ability to label compounds which are involved in processes such as cerebral blood flow, neurotransmitter receptor density and receptor binding (Gordon, 1999). SPECT technology may be of particular use for some researchers in the field who do not have access to a cyclotron (needed to form the radiolabelled tracer) as the activity of gamma-radiolabelled isotopes, used in SPECT, is sustained for much longer after initial formation than those used in PET (positron-emitting), prolonging their ability for transport (Gordon, 1999).

Magnetic resonance spectroscopy (MRS):

MRS relies on the principles of nuclear magnetic resonance (NMR) imaging. The principles of NMR will be described in full detail presently when we explore fMRI, another technique which capitilizes on the principles of NMR to explore brain function. Unlike fMRI, however, MRS is utilized to assess metabolite concentrations. Patients undergoing an MRS scan are not subjected to radioactivity, as in PET or SPECT, but rather magnetic fields are manipulated to yield a spectrum of frequency peaks for the metabolites. These "peaks" signify the NMR signal amplitude and are directly related to metabolite concentration (Stanley, 2002).

functional magnetic resonance imaging (fMRI):

Since the early 1990's fMRI has developed into a widely used tool for exploration of the brain, particularly with regard to the neural processing of information and in the investigation of neurocircuitry of neurological and psychiatric disease. To date, most psychiatric disorders have undergone substantial investigation using fMRI to gain insight into their psychopathology. Furthermore, fMRI has proven to be a useful tool in the study of therapeutic success in the treatment of psychiatric disorders. The remainder of the sections within this chapter will highlight the basic mechanisms underlying MRI and the relationship between the blood oxygen level dependent (BOLD) fMRI signal and brain function.

2.2 Principles of Nuclear Magnetic Resonance Spectroscopy (NMR)

An NMR, or MRI, signal is ultimately dependent on the nuclear properties of water and the chemical and physical environment which surrounds it (Howseman and Bowtell, 1999). Water, composed of two atoms of hydrogen and one atom of oxygen, is important in the creation of the NMR signal based on the fact that each hydrogen atom nucleus is composed of one proton with "spin". This nuclear "spin" essentially means that each hydrogen atom nucleus acts as a magnet, with a north and south pole (Buxton, 2002; Logothetis, 2002). When placed in an external magnetic field, these atoms align in the imposed magnet. However, these protons align, in the field, in two possible configurations. Slightly more than half of the protons align in a "low-energy state" while others align in a "high-energy state". In principle, the addition of another magnetic field, by applying brief radio-frequency (RF) energy, causes transitions between the two

nuclear spin energy states (Buxton, 1999). Following excitation, this system with time will return to thermal equilibrium, in the absence of another RF pulse. The NMR signal, which results from excitation, represents the difference between the energy absorbed and emitted during transition between the two energy states. Most commonly used in functional brain studies, BOLD fMRI optimizes this signal, by relying on blood flow changes inherent with brain activation and the magnetic properties which accompany changes in blood haemoglobin oxygenation.

2.3 The BOLD fMRI signal

The BOLD contrast fMRI signal is representative of alterations in a hemodynamic response, namely the change in deoxygenated and oxygenated haemoglobin concentrations occurring in the brain. The interpretation of this signal relies on the assumption that the flow of oxygenated blood to a region increases as neural activity is increased in the same region (Sheringham et al., 2002). It is this fundamental principle on which researchers rely to relate the fMRI signal to brain or neural activation. As an increase in oxygenated blood is recruited to the site of increased brain activity, it is used and is converted to deoxygenated blood. However, the demand for oxygen at the site is never large enough to use entirely the oxygenated blood in the region, resulting in excess oxygenated haemoglobin compared to deoxygenated haemoglobin in the region in which cerebral blood flow as been increased (Lubenberger, 1997). At this stage, changes occurring in the ratio between the deoxy and oxyhaemoglobin can be assessed using MRI technology. The production of the BOLD signal is dependent on the magnetic properties of haemoglobin, which varies accordingly to bound oxygen (Pauling et al., 1939). Deoxygenated haemoglobin is a paramagnetic compound (contains unpaired electrons

and is attracted to a magnetic field), while oxygenated haemoglobin is diamagnetic (contains no unpaired electrons) (Rajagopalan et al., 1995). Therefore, the presence of deoxy-haemoglobin results in an interaction with the applied magnetic field, resulting in magnetic field inhomogenities and a shortening of relaxation time (T2*). Oxygenated haemoglobin, which does not possess these same magnetic qualities, does not interfere with the magnetic field (Houseman and Boswell, 1999; Rajagopalan et al., 1995). Consequently, as cerebral blood flow increases the concentration of oxygenated haemoglobin relative to deoxygenated haemoglobin, the magnetic susceptibility produced by deoxygenated haemoglobin is reduced, resulting in a longer relaxation time (T2*), and essentially an increased local signal in regions of increased blood flow (Logothetis et al., 1999).

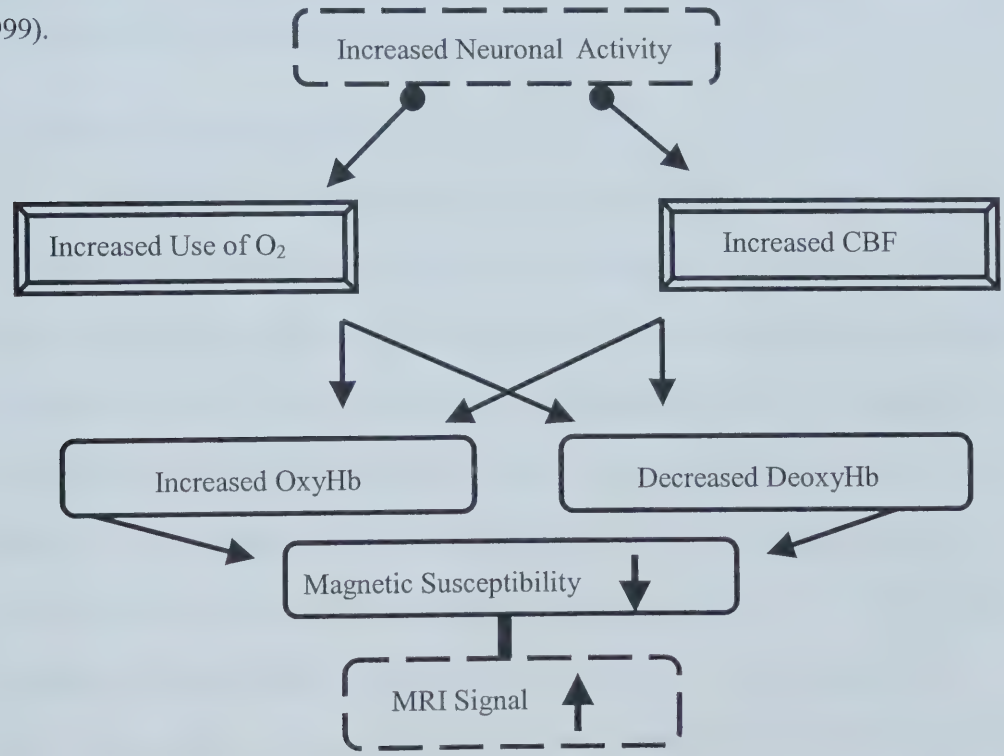

Figure 2-1
The principles of BOLD fMRI: The proposed sequence of events by which the MRI signal is altered by changes in neuronal activity, leading to a measurable BOLD fMRI signal difference in areas of increased activation.

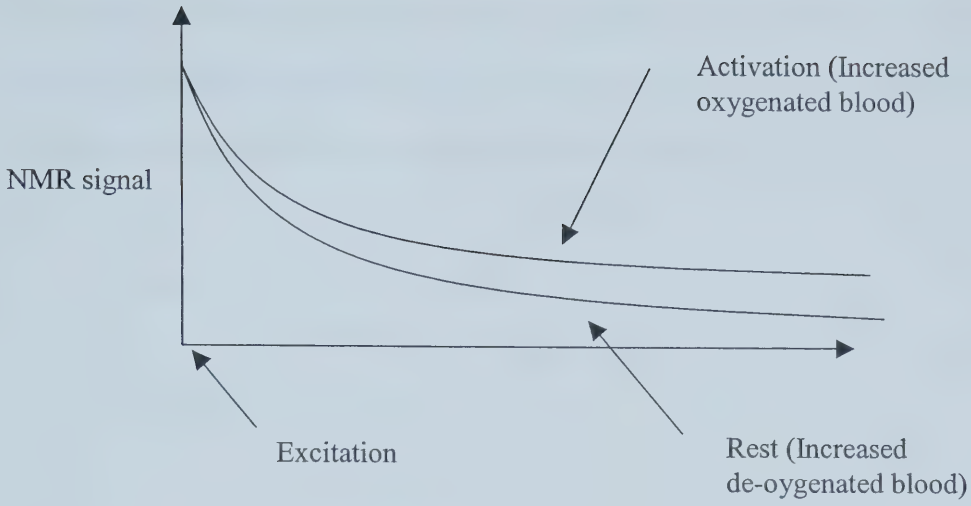

Figure 2-2
Alteration in the MRI signal due to the magnetic properties of deoxygenated and oxygenated haemoglobin, in activated and unactivated brain areas.

2.4 The haemodynamic response

The BOLD contrast response, measured by fMRI, is a direct measure of local deoxyhaemoglobin concentration. However, many factors such as increased blood volume, increased blood flow and increased oxygen consumption collectively affect the production of the BOLD signal in response to increased brain function (Howseman and Bowtell, 1999). Increased brain activation, following stimulation, is accompanied by a distinctive vascular response which is reflected in the BOLD contrast and is called the "haemodynamic response" (Willson, 2003; Howseman and Bowtell, 1999). Following stimulation, the initial haemodynamic response dips slightly for approximately one second. This is followed by an increase in the BOLD response, which peaks between 4 and 8 seconds after stimulation, and decays after stimulation is removed, returning to baseline following another slight response dip (Fransson et al., 1998; Houseman and

Boswell, 2002). The initial phase of the haemodynamic response reflects a hypo-oxygenation of a small brain region in response to neuronal stimulation. The second phase is indicative of hyper-oxygenation, which extends over a larger brain area and lasts longer than the first phase (Howseman and Bowtell, 1999).

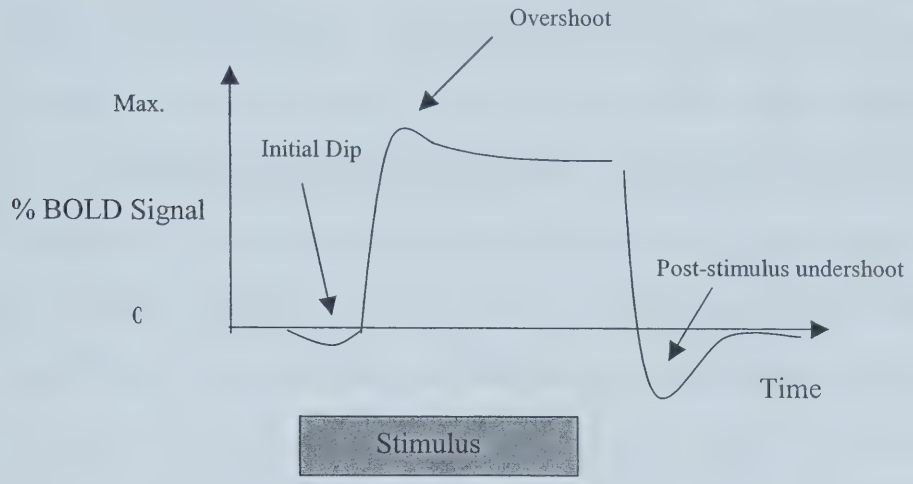

Figure 2-3
The influence of the haemodynamic or vascular response on the BOLD signal response, after neuronal activity increase due to stimulus presentation. Adapted from Jezzard 2001.

2.5 Interpretation of the BOLD signal

An understanding of how the BOLD fMRI signal relates to brain function is fundamental in the interpretation of functional imaging studies. We must explore the association between neural activity and the BOLD NMR signal, which only indirectly measures changes in regional blood flow and oxygen consumption. Moreover, we must consider the sources of oxygen consumption in regional brain areas, which may relate to and reflect neuronal activity.

The brain utilizes and requires a large amount of energy to sustain neural activity and therefore demands a continual supply of oxygen and glucose to drive oxidative

metabolism. The production of cellular energy, initiated by the conversion of glucose through glycolysis and the citric acid cycle, results from, in an aerobic environment, oxidative phosphorylation. In the brain, this energy produced is consumed by many processes, including neuronal spiking, action potentials and neurotransmitter cycling. It has been suggested that cellular energy in the brain is most significantly consumed by the reversal of Na^+ and K^+ ion fluxes, which accompany excitatory post synaptic potentials and action potentials (Attwell and ledecola, 2002). This remark would seem to indicate that a measure of energy consumption, based on glucose and oxygen metabolism, would reflect to a great extent synaptic activity, the major energy-demanding process in the brain. However, Attwell and ladecola (2002) have recently questioned if increased cerebral blood flow is significantly reflective of regional energy use. The authors suggest that while oxygen consumption during functional activation does correlate with changes in cerebral blood flow, factors other than oxygen usage most certainly play a role in determining cerebral blood flow to an area (Attwell and ladecola, 2002). The suggestion by researchers that fast glutamate signalling processes, not energy consumption, control local blood flow and therefore the haemodynamic response (the BOLD response) is important to note (Attwell and ladecola, 2002). Nevertheless, if functional activity is reflected by increased cerebral blood flow, which supplies oxygen needed for energy consumption and reflects neuronal signalling (Attwell and ladecola, 2002), then the BOLD fMRI signal should represent areas of increased neuronal activity. However, researchers still seek to ascertain what components of neural activity are reflected in the changes observed in the BOLD signal haemodynamic response in fMRI studies.

Even at its very best, BOLD fMRI resolution does not allow the acquisition of data from a voxel size which is small enough to contain only one neuron. Therefore, the BOLD response which is observed reflects the neuronal activity of many neurons in a population. Recently research has accumulated investigating the correlation between average neuronal response spike rate and the BOLD response.

The neuron spike or action potential

The resting membrane potential of a neuron represents the inequity of charged ions present in the cell and in the extracellular fluid which surrounds it. The membrane potential is of negative value, reflecting a more negatively charged cell cytoplasm compared to the fluid outside. This charge difference is caused by an excess of positively charged ions, such as Na^+, which accumulate on the extracellular side of the cell membrane (Koester, 1991). Increased permeability of ion channels, caused by neurotransmitter binding to post-synaptic receptors, allows the movement of ions such as K^+ (which is in excess inside the cell), Na^+ (which is in excess outside the cell), and Cl^- (which is in excess outside the cell) to move across the neuron cell membrane and results in depolarization or hyperpolarization of the cell. Increased permeability to Na^+ results in an increased Na^+ current into the cell, causing the cell to become more positive (depolarization). Increased permeability to K^+ ions and to Cl^- ions results in a more negative cell cytoplasm (hyperpolarization).

Depolarizations and hyperpolarizations which occur with temporal or spatial proximity sum. With adequate depolarization, the cell cytoplasm will become sufficiently positive to reach threshold membrane potential, and an action potential will be generated.

Once the all-or-none action potential is generated, the inward current of Na^+ allows the cell to become even more depolarized, producing the rising phase of the action potential (Koester, 1991). The closing of the Na^+ ion channels and the opening of K^+ ion channels results in the termination of the action potential, or neuron spike, and restores the membrane potential of the cell (Koester, 1991).

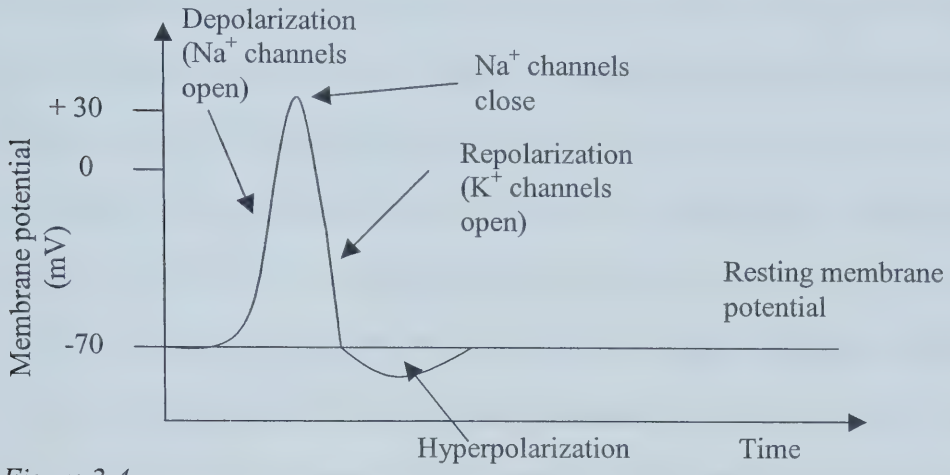

Figure 2-4
An action potential: The processes involved and the ionic changes which occur during the all-or-none firing of a neuron.

2.6 The neural basis of the BOLD signal

Two recent studies have investigated if a linear relationship exists between local neuronal firing rates and the BOLD signal. Rees and collegues (2000) carried out a study comparing average neuronal activity in monkey visual cortex V5 and the fMRI BOLD response (as % BOLD signal) in human V5. Neurons in the monkey V5 (or middle temporal visual area (MT)) and the V5 (or human middle temporal complex, MT+) equivalent in humans, have been shown to selectively fire in response to stimuli moving in a particular direction. For example, while some neurons respond mainly to a stimulus moving in an upwards direction, others fire preferentially in response to a stimulus

moving downwards or sideways (Britten et al., 1992; Heeger et al., 2000). These neurons are also characteristically sensitive to the amount of coherence among the moving stimuli (Heeger et al., 2000). Based on observations that the average neuronal response in monkey MT neurons increased in a linear fashion with coherence, and that the fMRI BOLD response in human MT+ cells also increased linearly with motion coherence, Rees et al. (2000) assessed the relationship between average neural activity and the BOLD signal. These researchers estimated that the proportionality constant between these two measures was 9 (1% BOLD signal reflects 9 spikes per second per neuron). This result is somewhat in contrast to those observed by Heeger and collegues (2000), who studied the relationship between the average neural activity in monkey visual cortex (V1) and fMRI BOLD signal response in human visual cortex (V1). The results of this comparison also demonstrated that the BOLD response was proportional to average neuronal activity (Heeger et al., 2000). However, in the latter study a much smaller proportionality constant of 0.4 was observed, indicating a much closer relationship between the percent change in BOLD signal and average neural firing rate (1% BOLD signal reflects 0.4 spikes per second per neuron). These recent findings strongly suggest that the BOLD signal is reflective of average neuronal activity. However, questions still remain to be answered in the exploration of this relationship. In particular, Heeger and collegues suggest that the BOLD signal response may also reflect changes in blood oxygen resulting from subthreshold neural activity, e.g. hyperpolarizations and depolarizations which do not generate action potentials.

2.7 Data acquisition in fMRI

The design of fMRI studies, monitoring activation changes over short stimulation blocks, demands fast data acquisition. Data is acquired, in most fMRI experiments, using an Echo Planar Imaging (EPI) sequence. The EPI sequence allows for rapid acquisition of brain images, such that a set of whole brain volumes can be imaged within 5 seconds (Jezzard and Clare, 2001). This is compatible with the goal of fMRI (i.e. to image changes occurring in the brain's haemodynamic response after a short-lived event or task). Furthermore, the EPI sequence allows successive recordings to be obtained with repetition of the stimulus (Gore, 2003). Single-shot EPI images are collected by applying one excitation RF pulse (Bandettini, 2001). However, data acquisition by EPI results in sacrificing, to some extent, the resolution of images to allow for acquisition over a shorter period of time (temporal speed) (Jezzard and Clare, 2001). The acquisition of fMRI images is also strongly influenced by the strength of the scanner's magnetic field (e.g. 1.5 Tesla, 3 Tesla, 4.7 Tesla). Increasing the strength of the magnetic field results in two effects on data acquisition. Magnetic susceptibility, which is increased as the magnetic field gets larger (the magnet stronger) can result in a larger amount of susceptibility artifacts (alterations in the T2 signal created by applying a static magnetic field to brain areas where the tissues or mediums have different magnetic properties, e.g. air/tissue interfaces), greater signal loss and distortions of the voxels, which will all impact data acquisition in a negative manner (Jezzard and Clare, 2001; Buxton, 1999). However, a greater magnetic field also allows for a greater signal-to-noise ratio, which is beneficial in the acquisition and analysis of fMRI data (Buxton, 1999).

CHAPTER 3

Neuroanatomy of Anxiety and Fear

It is well established, to date, that key neuroanatomical structures are involved in the experience of anxiety and fear. Recent advances in the field of neuroimaging have been drawn upon to gain further insight into the neurocircuitry underlying the experience of anxiety and emotion. This section will explore the functional neuroanatomy of anxiety and the research which has suggested the involvement of these specific brain structures in anxiety disorders.

3.1 The limbic system

The Limbic System:

The limbic system is composed of a group of gyri, namely the parahippocampal gyrus, the cingulate gyrus, and the subcallosal gyrus, which form the limbic lobe, and structures underlying the cortex, including the hippocampal formation, the nucleus accumbens, parts of the hypothalamus, orbitofrontal cortex and the amygdala (Kupfermann, 1991). The structures of the limbic system are highly interconnected with other sub-cortical structures and with the cortex (Kupfermann, 1991). It is valuable to outline the neuroanatomy and behavioral role of certain key cortical regions and structures which, by all accounts, play a role in the manifestation of anxiety and fear, before reviewing the literature regarding the neurocircuitry of anxiety disorders.

The Hippocampus:

The hippocampus is located on the medial surface of the temporal lobe. A curved structure, it is composed of three distinct zones; the dentate gyrus, the hippocampus proper, and the subiculum (Nolte, 1999). The entorhinal cortex is the primary structure delivering inputs to the hippocampus. Efferent fibers from the hippocampus are directed back to the entorhinal cortex and, through the fornix, extend to the cingulate cortex, the septal nuclei, the thalamus, the hypothalamus and the mammilary bodies (Nolte, 1999).

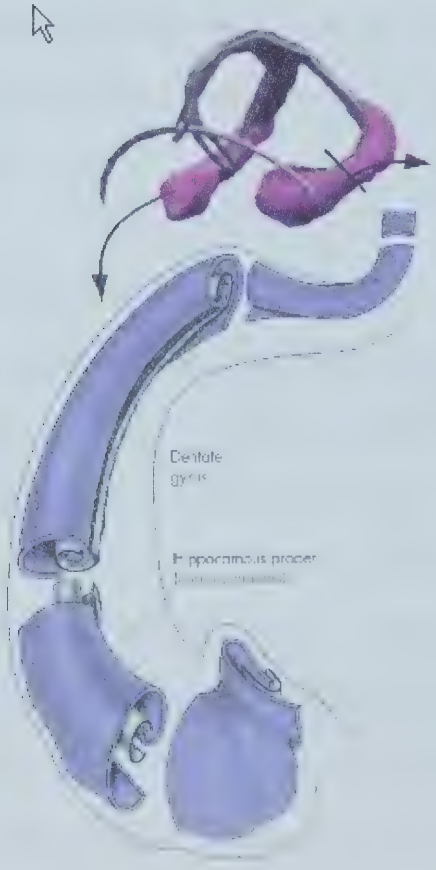

Figure 3-1
The structure of the hippocampus in the human brain. The three zones of the hippocampus; the dentate gyrus, the hippocampus proper and the subiculum. Copied with permission from Nolte (1999).

It is well established that the hippocampus plays an extensive role in memory formation and learning. The study of humans who had undergone removal of the medial temporal lobe initially called attention to the behavioral role of the hippocampus. Patients who suffered from bilateral hippocampal damage showed significant impairment in their ability to form new memories (Nolte, 1999). Although patients were unable to form new memories about a specific experience or store new factual information, their ability to learn skills and procedures remained intact (Nolte, 1999). Furthermore, studies have demonstrated that the neurons in the hippocampus are capable of long term potentiation (LTP) which may underscore the mechanism by which memories are formed in the hippocampus (Kandel, 1991).

Of importance, it has been suggested that the hippocampus plays an integral role in the development of contexual fear conditioning. Investigating the effect of dorsal hippocampal lesions in rats, researchers have observed a deficit in contexual fear conditioning (conditioning the rat to a novel environment where the novel contextual cues act as a conditioned stimulus) (Bast et al., 2001; Fanselow, 2000; Kim et al., 1993; Kim and Fanselow, 1992; Phillips and LeDoux, 1992). The same deficit, however, is not observed in the performance of a conditioned fear task where contexual cues do not act as the conditioned stimulus (Fanselow, 2000). Furthermore, Kjelstrup and collegues (2002) have suggested that the ventral hippocampus may play a role in unconditioned fear. In this study, rats subjected to lesions of the ventral hippocampus performed much differently than control sham animals or animals with dorsal hippocampal lesions. In the open-arm avoidance task, rats with ventral lesions failed to avoid the open arms of the

maze, unlike the sham and dorsal lesioned rats. Moreover, these rats displayed less defecation during a brightly-lit confinement task than those rats in either the sham or dorsal lesioned groups. These responses, to normally fear-provoking situations or stimuli, in the ventral lessioned animals are indicative of decreased levels of anxiety. These studies suggest that the hippocampus plays not only an integral role, which has been suggested, in memory and learning processes but also in the spatial processing of contexual cues in fear conditioning and even in the expression of some fear responses outside a contexual conditioning situation.

The Amygdala:

The subcortical amygdala, located dorsomedially in the temporal lobe, is composed of several nuclei. Afferent fibers to the amygdala from the cortex, the forebrain, the thalamus and the brainstem, input to the lateral, basolateral and central nucleus of the amygdala via four distinct routes (Davis, 1997; Nolte, 1999; Kupfermann, 1991). Projections from the amygdala extend through two pathways to the hypothalamus and the brainstem. The stria terminalis fibers output to the nucleus of stria terminalis, the nucleus accumbens and the hypothalamus. The ventral amygdalofugal pathway extends from the amygdalar nuclei to the hypothalamus, dorsal medial nucleus of the thalamus, and the cingulate gyrus (Kupfermann, 1991).

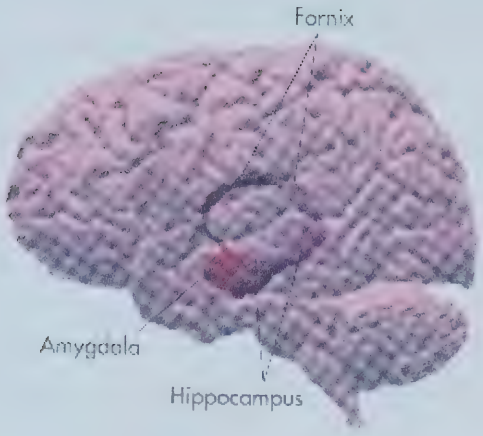

Figure 3-2
The location of the amygdala in the human brain. Copied with permission from Nolte, (1999).

Early studies in humans established that electrical stimulation of the amygdala results in feelings of anxiety (Chapman et al., 1954; Davis, 1997). Moreover, stimulation of the amygdala in animals can cause autonomic changes in blood pressure, heart rate, respiration and hormone release (Charney and Deutch 1996; Davis, 1997). An important discovery made many years ago highlighted the importance of the amygdala in the processing of fear in response to threatening stimuli. In 1939, Kluver and collegues observed that bilateral lesions of the anterior temporal lobes, including the amygdala and the hippocampus, in monkeys abolished the fear and behavior normally associated with a fearful stimuli (Kluver et al, 1939). Subsequent research demonstrated that while lesions of the amygdala elicited a characteristically abnormal emotional response to novel or threatening stimuli and reduced aggressive behavior, lesioning of the hippocampus resulted in impaired memory performance not accompanied by emotional response alterations (Zola-Morgan et al., 1991; Charney and Deutch, 1996). Furthermore,

researchers have observed an increased firing rate in the amygdala in response to threat and during fear conditioning (Charney and Deutch, 1996).

Additionally, the prefrontal cortex (PFC) shares extensive inhibitory connections with the amygdala (Quirk and Gehlert, 2003). It is well established that areas of the PFC influence affect and emotion. Moreover, researchers suggest that activation of the right-PFC predominates in the experience of negative affect (anxiety and fear), while the left-PFC is predominantly activated by positive affect (Davidson, 2002). Inhibitory interconnections between the PFC and the amygdala, both structures which are highly involved in affect, supports the notion that this circuit is important in the experience of anxiety and fear. Davidson and collegues (2000) have theorized that the PFC functions to terminate or influence the length of response of the amygdala to affective stimuli. In this regard, if there is a dysfunction of the inhibitory connections extending to the amygdala from the PFC, then amygdalar activity in response to emotional stimuli will be dysregulated and may become pathological (Davidson, 2002).

The Limbic Lobe:

The limbic lobe is composed of three gyri, namely the parahippocampal gyrus, the cingulate gyrus and the small subcallosal gyrus. The cingulate gyrus lies directly above the corpus callosum (Nolte, 1999). At the anterior end of the curved cingulate gyrus is the subcallosal area, while the posterior end of the cingulate gyrus curves into the temporal lobe and forms the parahippocampal gyrus (Nolte, 1999). These cortical areas are extensively implicated in recent neuroimaging studies of anxiety.

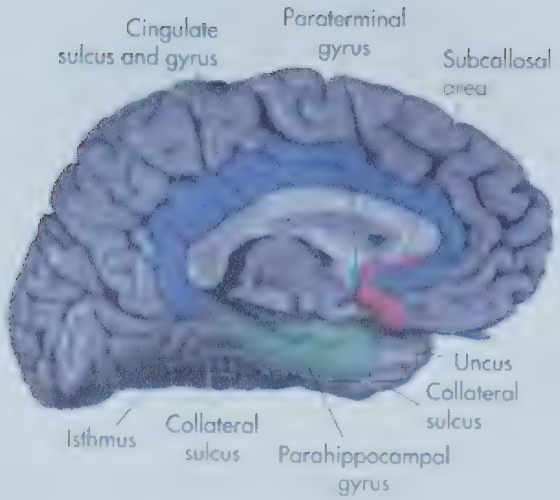

Figure 3-3
The structure of the limbic lobe: the location of the cingulate gyrus, the parahippocampal gyrus and the subcallosal gyrus (saggital view). Copied with permission from Nolte (1999).

The Insula:

The insula is not considered to be a component of the limbic lobe, but recent anxiety provocation studies have implicated this brain region in the manifestation of fear and anxiety. Therefore, a brief review of its location and neuroanatomy is beneficial. Hidden in the lateral sulcus, the insula is a piece of cortex overlaid by the parietal, temporal and frontal opercula (Nolte, 1999). The insular cortex is convoluted; generally it is composed of about 5 gyri and their sulci (Nolte, 1999).

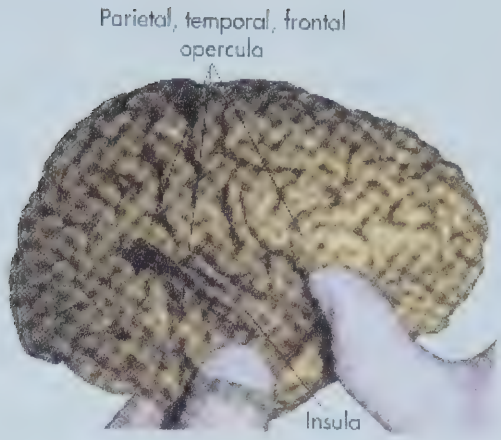

Figure 3-4
The location of the insula in the human brain. Copied with permission from Nolte (1999).

3.2 Functional neuroanatomy of anxiety and fear

To gain a better understanding of the importance and involvement of different brain structures in the processing of anxiety and fear, it is imperative to briefly discuss some findings from neuroimaging studies which have assessed brain activity and cerebral blood flow during anxiety and fear provocation. Therefore, at the outset of this section I will review studies which have investigated, in healthy individuals, the experience of anxiety and follow with a review of the findings in the neuropsychiatric population which have contributed to an understanding of the neuroanatomy of anxiety.

In order to investigate the experience of fear, LaBar and colleagues (1998) assessed brain activation in volunteers using fMRI to study a conditioned fear paradigm. In this paradigm, they assessed differences in brain activation when volunteers were presented with a cue (conditioned stimulus, CS+) accompanied by an aversive stimulus (unconditioned stimulus, US-) and a second cue (CS-) which was never followed by aversive stimulus application. They observed increased amygdala and periamygdaloid

cortex activation during the formation of the predictive relationship between the CS+ and the US, the acquisition of the conditioned fear response. Furthermore, they also observed amygdalar activation during the extinction of these trials (repeated presentation of the CS+ without the US). In both acquisition and extinction, the amygdala activation was primarily localized to the right hemisphere (LaBar et al., 1998). Moreover, numerous studies have found greater amygdalar activation in humans after presentation of fearful faces, compared to faces depicting other emotions (Davidson, 2002). In their 2001 fMRI investigation of 6 healthy adults, Thomas and colleagues observed increased left amygdala activity in response to the viewing of fearful faces, compared to neutral faces. This observation corresponds to earlier observations made by Whalen and collegues (1998) of increased amygdala activation during the viewing of masked fearful faces compared to the viewing of masked happy faces, in healthy subjects assessed by fMRI. Further evidence for the involvement of limbic structures in anxiety comes from a PET study conducted in 1998 by Fischer et al. which examined rCBF in a healthy female during the experience of an unexpected panic attack. During the panic attack, a decrease in rCBF was noted in the prelimbic cortex, the anterior cingulate cortex, the orbitofrontal cortex and the temporal cortex, all in the right hemisphere (Fischer et al., 1998).

In the past decade an extensive amount of research into neuropsychiatric disorders using functional neuroimaging has led to a greater understanding of the neural correlates of anxiety and fear. Many studies have investigated functional brain alterations in patients suffering from acute phobias, i.e. patients who experience anxiety reliably and immediately with exposure to a fearful stimulus. In 1997, rCBF of 14 subjects suffering

from a specific phobia (of snakes or spiders) was investigated after visual presentation of a phobic stimulus and after visual presentation of a neutral stimulus. During presentation of the phobic stimuli, the rCBF of patients was increased in the secondary visual cortex, compared to the neutral condition. Decreased rCBF was noted in the hippocampus, prefrontal, orbitofrontal, temporopolar, and posterior cingulate cortex (Friedrickson et al., 1997). These alterations in rCBF were accompanied by significantly higher subjective ratings of anxiety and distress, compared to reports obtained during the neutral stimulus condition (Fredrikson et al., 1997). In an earlier anxiety provocation study, Rauch and collegues (1995) studied rCBF alterations in 7 subjects with small animal phobia, after exposure to an individually tailored phobic stimulus and neutral stimulus. During the phobic trial, rCBF was increased in the left posterior orbitofrontal cortex, left insular cortex, left thalamus, right anterior cortex, anterior cingulate cortex and left somatosensory areas (Rauch et al., 1995). Altered rCBF in the frontal cortex, in response to anxiety provoking stimuli, is supported by the findings of Johanson et al. (1998), who demonstrated decreased frontal lobe rCBF bilaterally following anxiety provocation in spider phobics.

Assessement of these studies, which have collected data on the neural correlates of specific phobia, introduces some important considerations in evaluating the neuroanatomical structures involved in anxiety and fear. It has been suggested that increased neural activation in the secondary visual cortex, which is also characteristic in patients suffering from generalized anxiety disorder, represents increased alertness and attention during the experience of fear or anxiety (Wik, 1997; Friedrikson et al., 1997).

Furthermore, the observations by many researchers of increased rCBF or altered brain activation in the insular, orbitofrontal and cingulate cortices during anxiety provocation suggest that limbic, paralimbic structures as well as the PFC may be important brain areas in the manifestation of fear and anxiety. In addition, the fact that Paquette and collegues (2003) recently observed a normalization of activity in the dorsolateral prefrontal cortex and parahippocampal gyrus in patients with spider phobia following successful treatment with cognitive behavioral therapy (CBT) suggests that the limbic cortex and frontal cortex may play a significant role in the experience of anxiety prior to recovery.

Further insight into the neuroanatomy of fear has been gained by exploring neural dysregulations in patients with posttraumatic stress disorder (PTSD), who have, following a traumatic event, developed significantly disabling anxiety symptoms when exposed to certain stimuli. Most recently, Pissiota and collegues (2002) investigated the rCBF, during anxiety provocation, in 7 patients who suffered from PTSD subsequent to their participation in heavy combat and exposure to physical and emotional torture. Compared to the neutral condition, patients demonstrated increased rCBF in the premotor and motor cortices, the primary sensory cortex, the cerebellum, pariaqueductal grey matter and in the right amygdala (Pissiota et al., 2002). Earlier studies have also demonstrated an altered rCBF during anxiety provocation in patients suffering from PTSD. Two studies, involving women with PTSD subsequent to childhood sexual abuse, described different patterns of rCBF alteration during the anxiety condition, compared to the neutral condition. Increased rCBF in the orbitofrontal cortex and the anterior

temporal poles accompanied by a decrease in rCBF in Broca's area was reported by Shin et al. (1999) in these women. Bremner and collegues (1999), however, observed an increase in rCBF in the posterior cingulate cortex and prefrontal cortex as well as decreased rCBF in the right hippocampus (Bremner et al., 1999). In their review of the literature regarding the pathogenesis of PTSD, Pitman and collegues (2001) propose that activation of the amygdala and paralimbic structures in PTSD patients during anxiety provocation reflects the processing of negative emotions occurring during the recall experience (symptom provocation) and is involved in the increased autonomic response experienced by PTSD patients during the anxiety condition.

Neuroimaging studies have been extremely useful in the exploration of the neuroanatomy of anxiety. It appears that the limbic system as well as the prefrontal cortex are central in the manifestation of anxiety in healthy individuals and in individuals suffering from neuropyschiatric anxiety disorders. As a whole, these studies also support the dysregulation of many other brain areas, including the visual cortex, the thalamus and sensorimotor areas. Based on the findings obtained in patients suffering from simple phobia or PTSD, a disruption in the limbic system may be an important component influencing the pathophysiological anxiety experienced by these individuals. Furthermore, when one considers the extensive cortical connections extending from the limbic system, it is reasonable to assume that a greater cortical dysregulation is present, reflected by altered activity in many other brain areas during the experience of anxiety and fear.

CHAPTER 4

Neuroimaging in Social Phobia

The emergence of more advanced biomedical technologies and techniques over the last decade has greatly impacted on research exploring the biological basis of disease. Neuroimaging methods permit investigation in numerous avenues, including task-dependent brain activation, neurotransmitter binding sites and neuronal metabolism. In effect, neuroimaging studies have lent a greater understanding to the neurobiology and neurocircuitry of social anxiety.

4.1 Neuroimaging in Social Phobia

Initial neuroimaging studies conducted in SP patients were accomplished using magnetic resonance spectroscopy (MRS). Studies of SP using MRS, which evaluates metabolite changes based on frequency spectrum peaks, have produced conflicting reports. Initially, Davidson and collegues (1993b) investigated metabolite (creatine, choline, and N-acetylaspartate) alterations in 20 GSP patients (meeting DSM-III-R criteria) compared to healthy controls. In the caudate nucleus and the thalamus, creatinine and choline activity was significantly reduced in social phobics. Furthermore, the N-acetylaspartate signal-to-noise ratio was reduced in the caudate nucleus, the thalamus and in the white matter tracts examined, compared to healthy controls (Davidson et al., 1993). It has been suggested that decreased metabolite activity, as observed in this study, is indicative of neuronal loss (Agryropoulos et al., 2001). However, the authors caution against early interpretation of this research which pioneered

the field. Indeed, a repetition of this study was conducted by the same research group in 1997, and yielded slightly different results. In the investigation of 19 SP patients (assessed using the DSM-III-R) compared to healthy controls, by MRS, Tupler and collegues (1997) revealed significantly lower metabolite activity in cortical grey matter, as well as some altered activity in subcortical grey matter. In contrast to their previous findings, a minimal difference in white matter metabolite activity was observed between SP patients and healthy controls (Tupler et al., 1997).

Subsequently, single photon-emission computed tomography (SPECT) studies have been conducted in patients suffering from SP to investigate dysregulations in the brain of the dopamine system. In 1997, Tiihonen and collegues investigated, using SPECT, the density of dopamine reuptake sites in individuals with GSP. Using a cocaine labelled analogue, [^{123}I] 2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{123}I] β-CIT), to identify and establish the distribution of dopamine reuptake sites on the presynaptic cell membrane, 11 patients with GSP were compared to healthy controls. In this study, patients with SP had less reuptake of the marker than healthy participants, in the striatum; this observation represents a reduced density of dopamine reuptake sites in social phobics (a difference of 1.59 between healthy controls and social phobia patients in the ratio of dopamine reuptake sites in the basal ganglia to dopamine reuptake sites in white matter). Based on the findings from this study, Tiilhonen and colleagues (1997) suggest that patients with SP have fewer dopamine synapses and neurons in the striatum compared to healthy controls.

Exploring further the hypothesis of dopamine neuron deficiency in the basal ganglia in individuals with SP, Schneier and colleagues (2000) compared dopamine D_2 receptor binding potential in patients with GSP and controls. Using the radiotracer [^{123}I] iodobenzamide ([^{123}I] IBZM), it was found that subjects with SP had significantly decreased dopamine D_2 receptor binding potential in the striatum (Schneier et al., 2000). Furthermore, using positron emmision tomography (PET), the same deficit, decreased striatal dopamine D_2 receptor binding, has been shown in subordinate primates (Grant et al., 1998 in Mathew et al., 2001). These results support the notion of a striatal dopamine system dysregulation in the manifestation of SP.

More recently, the neurocircuitry (brain activation and blood flow) of SP has been explored. Several years ago, Stein et al. (1996a) examined if differences in baseline rCBF levels existed between 11 GSP patients and 11 healthy controls. No significant alteration in rCBF was observed in social phobics at baseline compared to healthy control subjects (Stein and Leslie, 1996). Subsequently, studies investigating brain activation and blood flow in psychiatric disorders have used anxiety provocation to scrutinize the relationship between anxiety and neural circuits. Using individual autobiographical audio scripts to induce recall of a social situation in which anxiety was experienced, and to induce recall of a relaxing situation (the control), Malizia and colleagues (1997) investigated, using PET scan, blood flow change in 6 SP patients. Subjective reports of anxiety were increased during the anxiety provocation task compared to the control relaxation task, and increased blood flow in response to social anxiety recall was

observed in the right dorsolateral prefrontal cortex and in the left parietal cortex (Nutt et al., 1998).

Furthermore, it appears that the evaluation of human facial expression of emotion may be altered in patients suffering from SP, evident from fMRI studies investigating brain activation following the presentation of visual slides of neutral, angry, fearful and contemptuous faces to social phobics. In the first of two studies, investigating altered brain activation in response to the viewing of facial emotion, described here, investigators presented 7 GSP patients with slides of neutral faces, an aversive foul odor and a neutral air puff. Birmbaumer and colleagues (1998) observed that in response to neutral faces, subjects with SP showed increased amygdala activation. This is of particular interest because previous research has shown that, in healthy controls, while the presentation of an aversive odor results in increased amygdala activation, the presentation of neutral faces does not (Zald et al., 1997). Even though the social phobic patients did not rate the neutral faces as harmful, the findings from this study may suggest that the amygdala becomes involved early on in the processing and evaluation of potentially anxiety provoking stimuli in patients with SP. In the second study, Stein et al. (2002a) have investigated brain activation in response to the presentation of angry, fearful, contemptuous, nonexpressive and happy faces in 15 GSP patients, compared to healthy controls, using fMRI. A comparison of brain activation when viewing each stimulus (fearful, angry, comtemptuous, nonexpressive) to brain activation when viewing happy faces revealed increased activation, in GSP patients, subcortically in the left amygdala, bilateral uncus, and the left parahippocampal gyrus during the presentation of angry and

comtemptuous faces (Stein et al., 2002). The same activation was not observed in healthy control subjects nor was it observed when comparing the activation during the viewing of fearful or nonexpressive faces with the viewing of happy faces in GSP patients (Stein et al., 2002).

Recently, Tillfors and colleagues (2001) conducted the first study in social anxiety disorder patients (n = 18), using an actual public speaking paradigm to investigate rCBF using PET. In this study, a greater increase in rCBF in subjects with SP (compared to controls) was observed, during public speaking versus private speaking, in the right amygdala (Broadmans area 34) and hippocampus. During public speaking, decreased rCBF, compared to controls, was observed in these patients in the insular cortex and in the right temporal pole. SP patients also had a greater decrease in rCBF during public speaking in the orbitofrontal cortex. In controls only, Tillfors and colleagues report increased activation in bilateral perirhinal cortex and the retrosplenial area. These results clearly demonstrate a significantly altered pattern of subcortical and cortical activity in patients with SP, when exposed to fearful stimulus provocation and are in keeping with theoretical expectations. Patients in this study met the DSM-IV criteria for SP, but it seems that the group was a mix of individuals suffering from the specific and generalized forms of the disorder. A further extension of this study aimed to identify if successful treatment of SP with citalopram, an SSRI, or cognitive behavioral therapy (CBT) normalized the rCBF observed in response to the public speaking task. Indeed, in responders (both CBT and citalopram groups) there was a significantly decreased rCBF in the amygdala (bilaterally), right hippocampus and temporal cortex (Furmark et al., 2002). This result may indicate that treatment success relies partly on a normalization of

brain activation in the temporal lobe, the amygdala and other interrelated and interconnected brain areas.

The data collected in neuroimaging symptom provocation studies in anxiety disorders, using PET and fMRI possess many conflicting reports of brain alterations. The limitations of each of these neuroimaging techniques may contribute to the inconsistency of the results. Most importantly, in discussing fMRI, one must consistently be aware that the BOLD signal only indirectly reflects the coupling of neural activity in the brain to the haemodynamic response (Gore, 2003). Moreover, the intensity of the BOLD signal is determined not only by the changes of blood flow to tissue but also by the changes in oxygen content of the tissue (Gore, 2003). This suggests that fMRI is not directly able to measure rCBF, as PET. However, the increased spatial and temporal resolution of fMRI (although still unable to pinpoint activation of specific brain nuclei),compared to PET, as well as the ability to measure, through haemodynamic coupling, areas of increased brain activation; have contributed greatly to our understanding of numerous brain functions.

4.2 Objectives

The main objective of the study detailed in this thesis was to explore, using fMRI, the neuroanatomical correlates of public speaking anxiety in individuals suffering from SP. Our aim was to examine with fMRI alterations of brain activation during public speech (with observers present) in individuals diagnosed with generalized social anxiety disorder (or GSP) and HC subjects using private speech (without observers present) as a control procedure.

B. MATERIALS AND METHODS

Chapter 5

Materials

5.1 Instrumentation

MRI Scanner:

All fMRI images were obtained on a Siemens Sonata 1.5 Tesla Scanner located at the University of Alberta Hospital Nuclear Magnetic Resonance (NMR) Centre.

fMRI Analysis Software:

Analysis of fMRI images was accomplished using Statistical Parametric Mapping (SPM99) software (Wellcome Department of Cognitive Neurology, London, UK). This software was run using MATLAB 6.5 Release 13.0 (licensed by University of Alberta Computer Network Support, 2003). Post-analysis activation images were superimposed onto brain templates for presentation using MRIcro version 1.36. Following analysis with SPM99, coordinates of interest were converted by Tailarach Daemon (University of Texas Health Science Center at San Antonio, ric.uthscsa.edu/projects/talairachdaemon.html) to identify proximity to brain neuroanatomical structures and Broadman's areas. All software programs were installed and run on an Intel (R) Pentium 4, 2.40GHz at 1,048,096KB RAM (registered by the Faculty of Medicine and Dentistry University of Alberta, 2003).

5.2 Subjects

Social Phobia Patients:

Fifteen subjects (7 males and 8 females; mean age ± SD = 29.6 ± 6.40) suffering from generalized social phobia were recruited by means of advertisements. All potential research subjects underwent a telephone screen prior to the first visit to identify any potential reasons for exclusion from the study and willingness to participate. Written informed consent of participation was obtained from subjects after they had been fully explained the procedures and their involvement in the study. Subjects were diagnosed as meeting criteria for GSP as defined by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association, 1994) by administration of the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1995). All patients were free of other current comorbid Axis I disorders at the time of the study. Five subjects had a prior history of major depression, and one a prior history of panic disorder and agoraphobia.

The severity of social anxiety and the extent to which subjects feared public speaking was assessed using the Liebowitz Social Anxiety Scale (LSAS) and the Personal Report of Confidence as a Speaker (PRCS), respectively. The mean scores ± SD for patients with GSP were LSAS = 84.60 ± 13.59 and PRCS = 24.73 ± 3.71. A mean rating of above 80 indicates that the GSP patients suffered from moderate to severe SP. The Beck Depression Inventory (BDI) and the Hamilton Anxiety Scale (HAM-A) were also administered to patients (BDI= 8.27 ± 6.40; HAM-A = 11.73 ± 5.84). One female patient suffering from GSP did not return for the MRI scan.

Four subjects (3 males and 1 female) diagnosed as suffering from specific social anxiety disorder of public speaking were also recruited by means of advertisement for the study (LSAS = 52.50 ± 16.42 ; PRCS = 27.50 ± 1.73). However, due to this small

sample size, and the unknown commonalities in neurocircuitry which exist between the two disorders, we have chosen not to include these patients in the analysis. Moreover, we have eliminated one male GSP patient in our analysis based on the fact the he was unable to carry out the private or public speaking tasks.

Healthy Controls:

Fourteen subjects (7 males and 7 females; mean age ± SD= 26.21 ± 5.34) were recruited by means of advertisements for the study. All potential research subjects underwent a telephone screen prior to the first visit to identify any potential reasons for exclusion from the study and willingness to participate. Written and informed consent of participation was obtained by subjects after they had been fully explained the procedures and their involvement in the study. Subjects were deemed healthy controls based on a subjective report of comfort in public speaking and based on the fact that all had extensively been exposed to public speaking situations. Healthy controls (HC) also completed the LSAS and PRCS to verify their comfort level in social settings (LSAS = 11.14 ± 10.24; PRCS = 2.86 ± 1.66). Depression and anxiety ratings on the BDI and HAM-A were also obtained (BDI = 1.86 ± 2.07; HAM-A = 3.57 ± 2.65) in these subjects. None of the HCs had any past or present history of Axis I psychiatric disorders, and none had a family history of diagnosed first order relatives with social anxiety disorder.

Inclusion and exclusion criteria for all subjects (SP and HCs):

All subjects were free of the use of psychotropic medications for at least 2 weeks (5 weeks for fluoxetine) prior to participation. Subjects were excluded if pregnant or

suspected to be pregnant (all subjects underwent a urine pregnancy test), or if they had a history of head injury (in which they reported unconsciousness), head trauma, or seizures. Furthermore, subjects were excluded from participation for safety reasons with regard to exposure to the magnetic field if they had any metal in their body (e.g. metal fragments, metallic prosthetic devices, pacemakers, aneurysm clips or other metal implants or a history of employment as a welder). The medical history of participants was obtained to aid in identifying any possible safety risks of this nature, and participants were required to sign a second consent form prior to imaging to ensure the absence of metallic objects in the body. Subjects were also required to refrain from the use medications affecting cerebral blood flow 2 weeks prior to the MRI scan and subjects were excluded if they suffered from claustrophobia or hypertension. Participants were also asked to refrain from the use of caffeine, alcohol or cigarettes for 12 hours prior to the MRI.

All female patients were scanned during the follicular phase (FP) of their menstrual cycle (days 1-14). As menstrual hormones are at their lowest levels in the FP, we elected to scan women at this time only to minimize any hormonal effects which may have affected results obtained during the scan.

5.3 Social phobia scales

Leibowitz Social Anxiety Scale (LSAS):

The LSAS is a popular tool used in the assessment of fear, anxiety and avoidance of social situations in the social phobic population. In this clinician administered scale, patients are asked to rate their degree of fear or anxiety and frequency of avoidance of 24 social and performance situations (Safren et al., 1999). Previous studies have confirmed

the validity of the LSAS in comparison to other scales used in social anxiety such as the Social Interaction Scale, Social Phobia Scale, and the Social Avoidance and Distress Scale (Heimberg et al., 1999). Moreover, the LSAS displays high internal consistency and discriminant validity with the HAM-A and BDI. The LSAS explores social anxiety in four categories, namely social interaction, public speaking, observation by others, and eating and drinking in public (Safren et al., 1999). Recent data suggest that a cutoff LSAS of 30 (total score) is most appropriate for making a diagnosis of SP, and a cutoff of 60 as a total score is reliable in the diagnosis of generalized social anxiety disorder (Mennin et al., 2002).

Personal Report of Confidence as a Speaker:

The PRCS scale is a 30 item questionnaire which asks for true or false responses based on experience speaking in front of an audience. It is scored according to a legend, with a resulting score of 0 representing no fear of public speaking, and a response of 30 representing severe fear of public speaking (Phillips et al., 1997). Studies which have used the PRCS in allocating subjects to public speaking anxiety groups have used varying cutoffs, such as a score of 16 or 17 (Phillips et al., 1997).

CHAPTER 6

Methods

6.1 Study design

This study design involved two visits to the University of Alberta Hospital, following an initial telephone contact.

Visit 1 (V1): This visit was approximately 2 to 3 hours in length for each subject. The first visit was composed of a diagnostic interview for all patients. At this visit patients also completed a consent form, following detailed explanation of participant involvement in the study. Eligibility and a diagnosis of social anxiety disorder, or SP, was established by the administration of the SCID for DSM-IV. Healthy volunteers also underwent this diagnostic interview to ensure eligibility and the absence of present or past psychiatric axis I disorders. A battery of psychometric questionnaires, ie. the LSAS, PRCS, BDI, HAM-A, was also administered during this visit. A medical and psychiatric familial history was also recorded. At this visit, female patients underwent a urine pregnancy test, and a "sitting" blood pressure (BP) was obtained for both female and male participants. After this visit and confirmation of eligibility, participants were booked for the second visit, and a booking of one hour of the Siemens 1.5 T MRI scanner was entered at the Nuclear Magnetic Resonance (NMR) Centre, University of Alberta Hospital.

Visit 2 (V2): Participants returned for their second visit approximately 15 minutes before the reserved magnet time. The instructions for this visit were reiterated at

this time and it was assured that all patients had refrained from caffeine, alcohol and cigarettes for 12 hours prior to the visit. Prior to entering the scanner, participants completed a second consent form which detailed MRI exclusion criteria (e.g. metal in the body). Each subject was allowed a 5 minute preparation period prior to entering the scanner to gather some thoughts towards making a speech about a vacation or travel experience, although they were not allowed to write out this speech fully. While in the scanner, the participants were required to present a speech one and a half minutes in length, based on this preparation, a total of 4 times, remaining as close as possible to the original each time. Subjects were required to present this speech twice while they were alone in the scanner room and twice in front of a set of observers (5-10 individuals).

Paradigm: Presentation of the Speech

After the preparation period, participants were placed lying on his/her back inside the scanner and were instructed to lie as still as possible. A mirror was placed above the participant's head, allowing him/her to see at their feet the outside of the scanner where the observers would be present. Once comfortable lying in the scanner, the private/public speaking paradigm was begun. After 45 seconds of rest, subjects were instructed to begin the speech for the first time. Participants were instructed to stop this speech after 1.5 minutes. This was followed by a rest period of approximately 2 minutes without speaking, and then subjects were instructed to begin speaking for the second time. Yet again, subjects were stopped after 1.5 minutes of speaking. The second speech was followed by a 3.5 minute rest period. The third speech was then initiated and stopped after 1.5 minutes, and subjects rested for approximately 2 minutes. Subjects were at last instructed to give their fourth, and final, speech of 1.5 minute duration. For all subjects

in this study, the first two speeches took place while the subject was alone in the scanner room (private speech) and the third and fourth speeches took place with an audience (public speech). See *Figure 6-3* for a visual depiction of the public speaking paradigm.

Paradigm: fMRI Imaging

Brain images were collected on the 1.5T Seimens Sonata scanner using a single shot EPI gradient echo sequence (TR=4500ms, TE=50ms, 64x64 matrix, 220 FOV) to acquire 30 contiguous 4mm slices to cover the entire brain. Images were collected for 45 seconds prior to the start of each speech, and during the first 45 seconds of each speech. This image collection protocol was repeated for each of the 4 speeches, resulting in a set of baseline images for each speech and a set of activation images for each speech. See *Figure 6-3* for a visual depiction of the publics speaking paradigm and fMRI scanning protocol.

Paradigm: Behavioral Measures

During the second visit of the study, the scanning visit, behavioral measurements of anxiety were obtained for each patient. Two different subjective measures were collected during this visit.

Visual Analogue Scale (VAS): Subjective reports of anxiety were collected throughout the MRI visit, using a 10 point anxiety scale. Subjects responded to the statement "*Please rate your present level of anxiety*" choosing one number on the scale that best represented their level of anxiety at that time (a score of 0 represented minimal anxiety, a score of 5 represented moderate anxiety, and a score of 10 represented maximal anxiety). This scale was administered to participants 15 minutes prior to entering the scanner (VASbas), 5 minutes before beginning the paradigm (while in the scanner)(VAS-5min),

45 seconds prior to beginning each speech (VAS- pre-speech 1, pre-speech 2, pre-speech 3, pre-speech 4), and during the rest period connecting the second and third speeches (between the last private speech and the first public speech; VASrest).

*Please rate your **present** level of anxiety on the scale below:*

Mild Moderate Severe

Figure 6-1
Visual analogue scale: present level of anxiety (VAS).

Visual Analogue Scale Change in Anxiety (VAS-change): This 10 point scale represents the increase in anxiety participants experienced during the presentation of each speech. Participants were instructed to pick the number that best reflected their answer to the statement, *"Please rate the change in your level of anxiety from before giving your speech, this time, to the maximum level experienced while giving your speech"* provided that a score of 0 represented a minimal increase in anxiety and a score of 10 represented a maximal increase in anxiety. This scale was completed following each speech (VAS-change; speech 1, speech 2, speech 3, speech 4).

*Please rate the **change** in your level of anxiety from before giving your speech to the maximum level you experienced while giving your speech:*

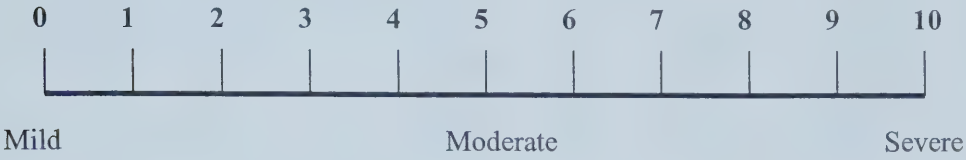

Figure 6-2
Visual analogue scale: change in level of anxiety (VAS-change).

We have administered both scales (VAS, VAS-change) in the time periods between the presentation of the speeches and between the fMRI imaging blocks. It is not feasible to ask or to request an answer during the periods in which the subject is speaking or scanning is underway. See *Figure 6-3* for a visual depiction of the public speaking paradigm and administration of the behavioral scales.

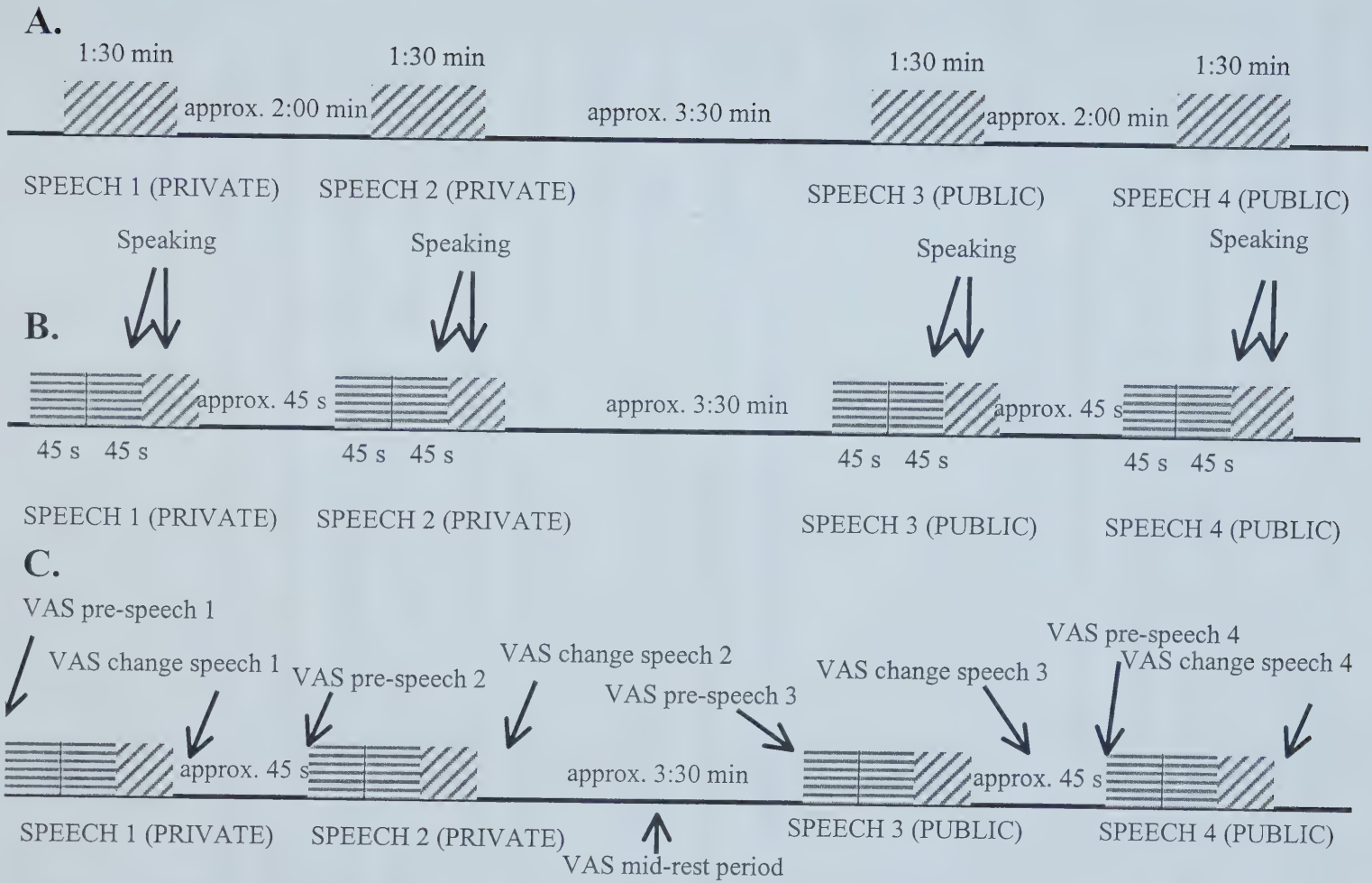

Figure 6-3 "fMRI of a public speaking task" paradigm design: presentation of the speech (A), fMRI imaging (B), and behavioral scale administration (C).

6.2 Statistical analysis

(see Appendix 1 which details the exact steps followed for analysis using SPM99 software; and a sample data set)

Spatial pre-processing and statistical analysis of MRI images were preformed using SPM99 software. First, the raw images obtained on the Siemens 1.5 T scanner were transferred to a Pentium 4 running off a Windows 2000 platform. A set of 4 pre-processing steps preceded the statistical analysis.

Pre-Processing:

a. Reorientation: All images were reoriented along the x and y axis (flipped) to accommodate for the orientation of template images which are used in the SPM99 software. All images were also adjusted to the fit of this template in planes of rotation (pitch, roll, yaw).

b. Realignment: To correct for subject motion during the acquisition of images, all the images were realigned to the first T2* image obtained in each "session" (if one considers each set of images obtained in each condition as a session). For example, for the first speech, baseline images were realigned to the first image obtained prior to the start of speaking, and the activation images were realigned to the first image obtained during the first speech. This realignment was carried out for each speech, for baseline and activation images.

c. Normalization: The images were then normalized into an ideal standard template space using a 12-parameter affine normalization and a nonlinear estimation of deformations.

d. Smoothing: Finally, the images were smoothed or "blurred" with an 8-mm full width at half maximum (FWHM) Gaussian kernel to increase the signal-to-noise ratio (where signal equals BOLD response to stimulation and noise equals random intensity changes in the BOLD response which occurs without stimulation) and to permit further statistical analysis.

Statistical Analysis (SPM99):

A series of steps were used to carry out multiple statistical comparisons of the acquired fMRI data.

Step 1: *Mean image creation*

For each patient, mean images during each speech were compiled from the images taken during the speaking tasks (speeches 1, 2, 3, and 4) to yield four mean images of speaking, one for each speech. Mean images were also compiled from images taken before each speech (speeches 1, 2, 3 and 4) to yield four baseline mean images, one for each speech. Furthermore, four mean images were created from the above to yield, for each subject, a combined mean image during private speaking (speeches 1 and 2), a combined mean image during public speaking (speeches 3 and 4), a mean image at "baseline" prior to private speaking (speeches 1 and 2), and a mean image at "baseline" prior to public speaking (speeches 3 and 4), where "baseline" refers to the data collected for 45 seconds prior to the initiation of speech (see *Figure 6-4).*

Step 2: *t-Tests*

Paired t-test were carried out for the following conditions:

a. Private speaking vs. Baseline private speaking (for GSP group and HC group) (see *Figure 6-5*)
b. Public speaking vs. Baseline public speaking (for GSP group and HC group) (see *Figure 6-5*)
c. Public speaking vs. Private speaking (for GSP group and HC group) (see *Figure 6-6*)

The threshold p-value was set at 0.001 for all paired t-tests.

Analysis of VAS and VAS-change:

An analysis of repeated measures was carried out to evaluate the main time effect for the VAS responses and the VAS-change responses for each group, GSP and HC. t-Tests were performed to assess, in each group, the change in anxiety from baseline (VAS-5min) to pre-private speech (VAS pre-speech 1 and VAS pre-speech 2), and the change in anxiety from rest (VASrest) to pre-public speech (VAS pre-speech 3 and VAS pre-speech 4)). Statistical analyses of the change in anxiety which took place from before giving the speech to the maximum intensity experienced during the speech (private and public, VAS-change) were also assessed. Statistical significance was set at the 5% level for within-group analysis of all scales.

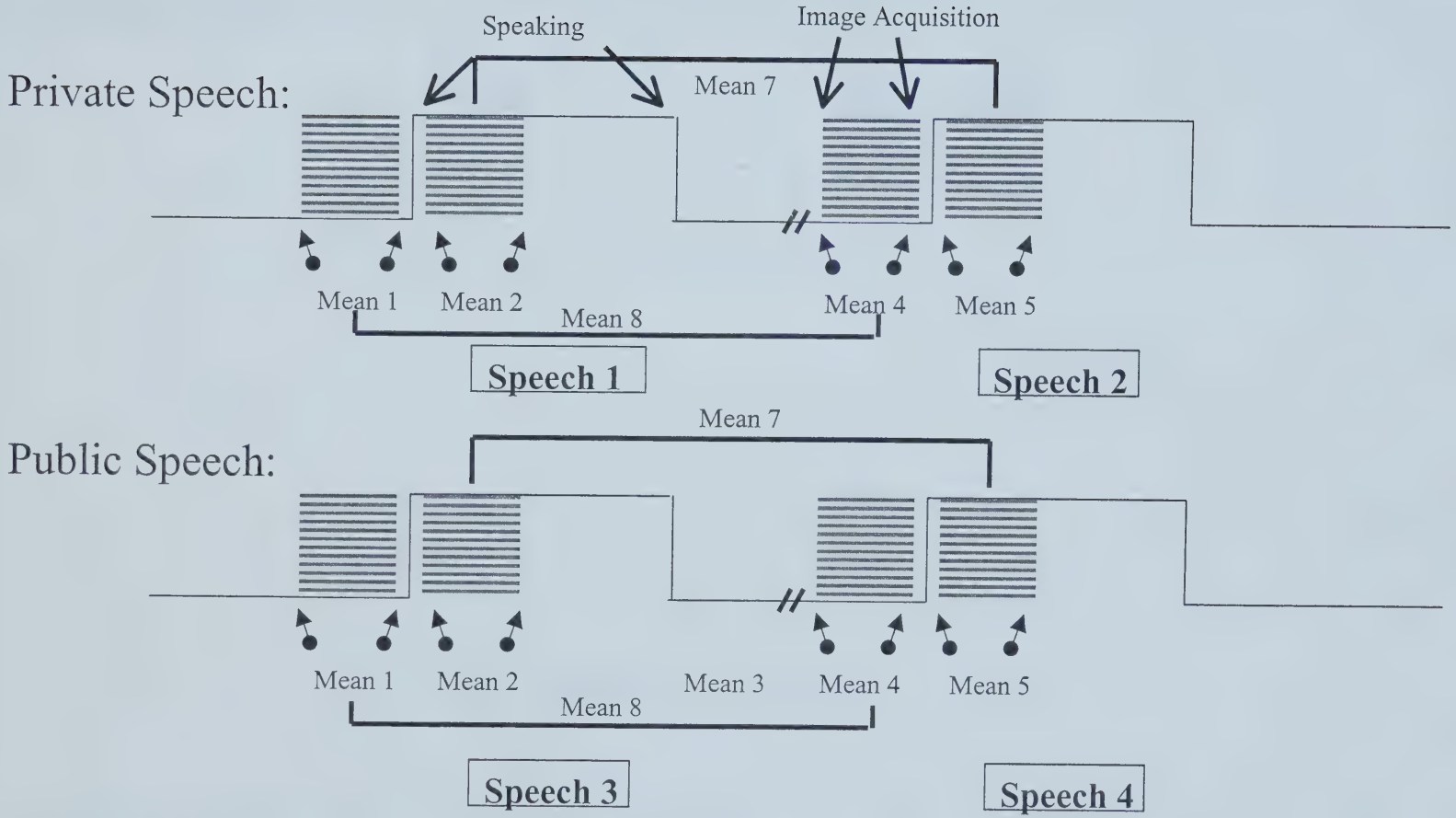

Figure 6-4 Creation of mean images for statistical analysis using SPM99.

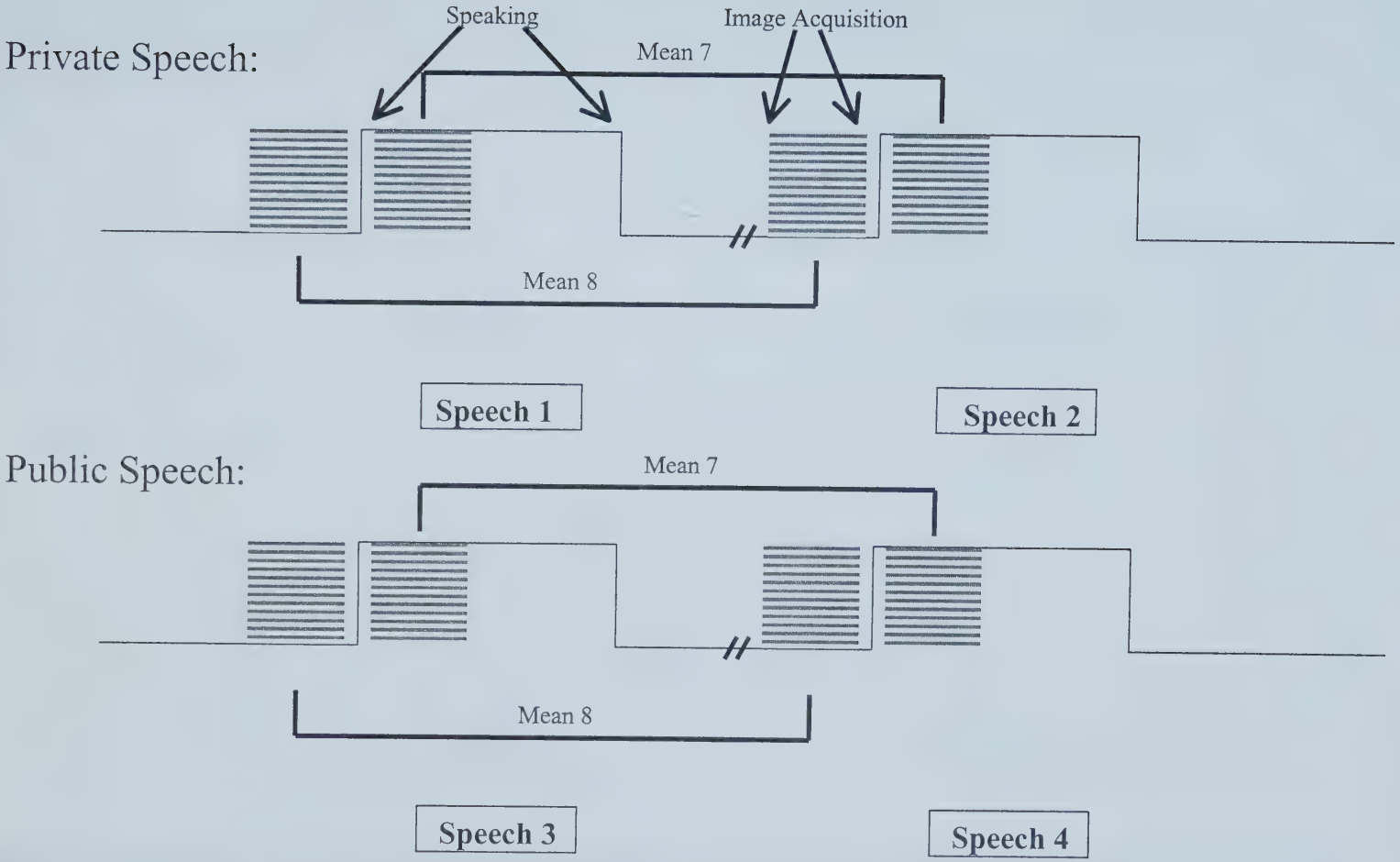

Figure 6-5 The design of the statistical comaprison (t-tests) performed for private or public speaking conditions (mean 7) versus baseline conditions (mean 8).

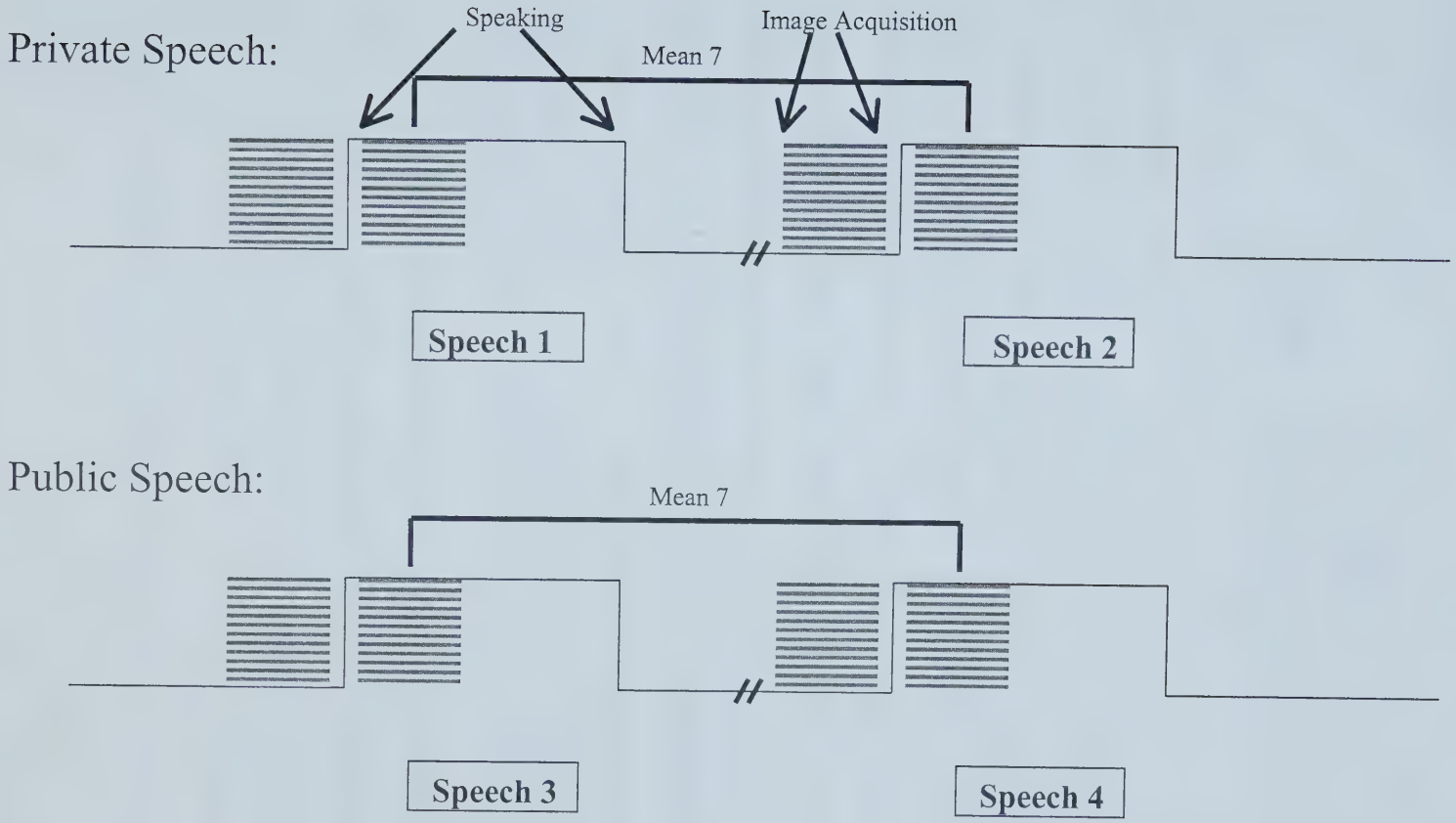

Figure 6-6 The design of statistical comparison (t-tests) performed for private versus public speaking conditions(private mean 7, public mean 7).

CHAPTER 7

fMRI Analysis of Brain Activation

7.1 Speaking vs. Baseline

GSP subjects (n =13)**:**

Patients with GSP demonstrated increased activation in the private speaking condition, relative to baseline of private speech, in the left and right precentral gyri (BA6), the left and right cerebellum, the left superior frontal gyrus and the left middle temporal gyrus (BA21) (*see Figure 7-1; Table 7-1*). In the public speaking condition, relative to baseline of public speech, increased BOLD signal was observed in the left and right precentral gyri, left and right cerebellum, the right cingulate gyrus, left and right superior temporal gyri, right fusiform gyrus, right middle temporal gyrus, and right thalamus (*see Figure 7-2; Table 7-2*). All areas met a thresholding p-value of 0.001 uncorrected.

HC subjects (n = 14)**:**

Nonphobic HCs demonstrated increased BOLD signal in the private speaking condition relative to baseline of private speech, in the left and right precentral gyri (*see Figure 7-3; Table 7-3)*) and in the public speaking condition, relative to baseline of public speech in the left and right precentral gyri, and in the right temporal lobe (sub-gyral) and left frontal lobe (sub-gyral) (*see Figure 7-4; Table 7-4*).

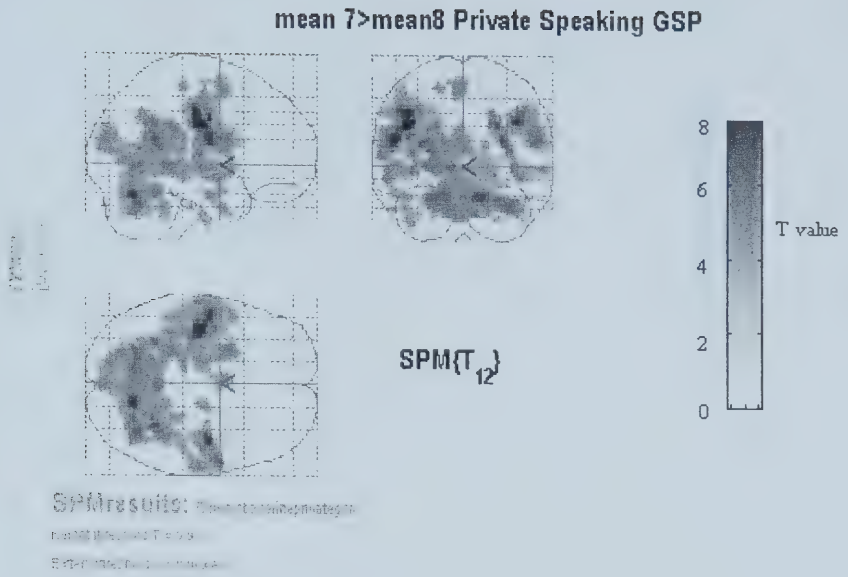

Figure 7-1
Brain areas of increased activation during private speaking versus public speaking baseline condition, in patients with GSP. Darker areas of gray represent areas of greater activation and of high T-value (corresponding to the gray scale).

Brain Regions Activated	Talairach coor. (mm)			Z-Score	p value (cluster unc)
	x	Y	z		
L Precentral gyrus	-40	-14	30	5.86	0.000
R Precentral gyrus (BA6)	40	-6	32	5.28	0.000
R/L Cerebellum	16 , -24	-63 , -61	-15 , -17	5.16	0.000
R Parietal lobe (sub-gyral)	26	-47	32	4.64	0.000
R Temporal lobe (sub-gyral)	38	-37	0	4.32	0.000
L Superior frontal gyrus	-4	6	51	4.01	0.002
L Middle temporal gyrus(BA21)	-44	1	-27	3.70	0.016

Table 7-1
fMRI analysis of GSP patients. Areas of increased activation in GSP patients during private speaking versus private speaking baseline condition. Uncorrected p-value threshold set at 0.001.

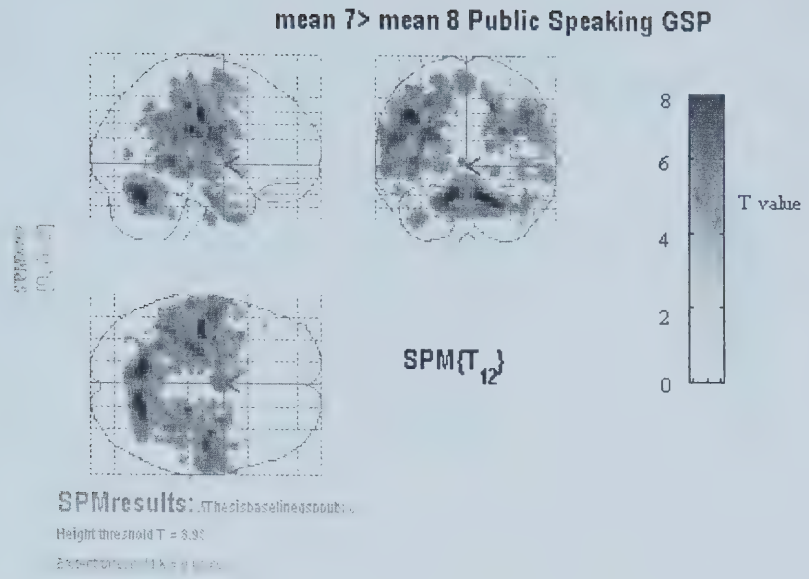

Figure 7-2
Brain areas of increased activation during public speaking versus public speaking baseline condition, in GSP patients. Darker areas of gray represent areas of greater activation and of higher T-value (corresponding to gray scale).

Brain Regions Activated	Talairach coor.(mm)			Z-Score	p value (cluster unc)
	x	Y	z		
L Precentral gyrus	-42	-16	36	5.69	0.000
R/L Cerebellum	16 , -14	-63 , -61	-17 , -17	5.44	0.000
R Precentral gyrus	44	-12	37	4.78	0.000
R Cingulate gyrus	20	-23	42	4.74	0.000
L Superior temporal gyrus (BA38)	-38	10	-34	4.21	0.003
R Superior temporal gyrus	57	-14	-1	4.18	0.000
R Fusiform gyrus	44	-9	-23	4.11	0.001
R Middle temporal gyrus	46	5	-25	3.49	0.001
R Medial globus pallidus	20	-10	-5	3.88	0.022
R Thalamus	14	-16	1	3.21	0.022

Table 7-2
fMRI analysis of GSP patients. Areas of increased activation in GSP patients during public speaking versus public speaking baseline condition. Uncorrected p-value set at 0.001.

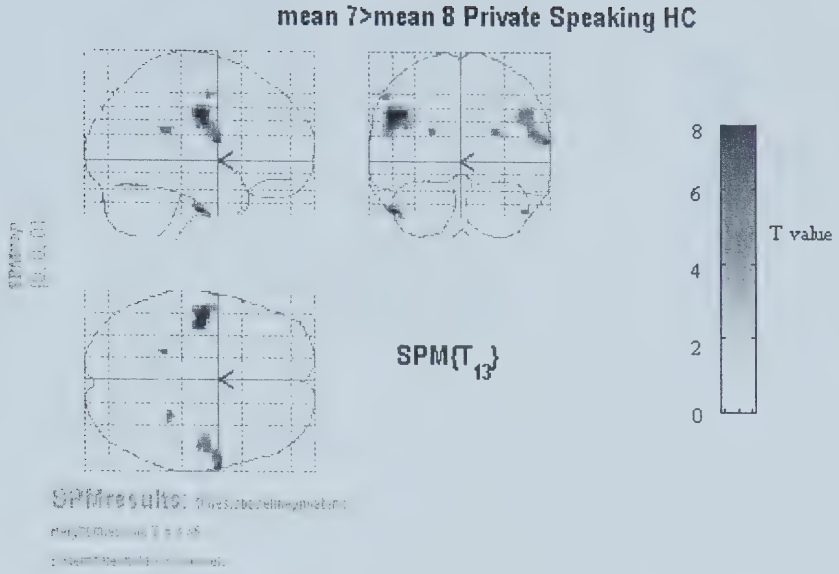

Figure 7-3
Brain areas of increased activation during private speaking versus private speaking baseline condition, in HC subjects. Darker areas of gray represent areas of greater activation and of higher T-value (corresponding to the gray scale).

Brain Regions Activated	Talairach coor. (mm)			Z-Score	p value (cluster unc)
	x	y	z		
L Precentral gyrus	-50	-8	34	4.32	0.001
R Precentral gyrus (BA6)	63	1	15	3.89	0.004

Table 7-3
fMRI analysis of HC subjects. Areas of increased activation in HC subjects during private speaking versus private speaking baseline condition. Uncorrected p-value set at 0.001.

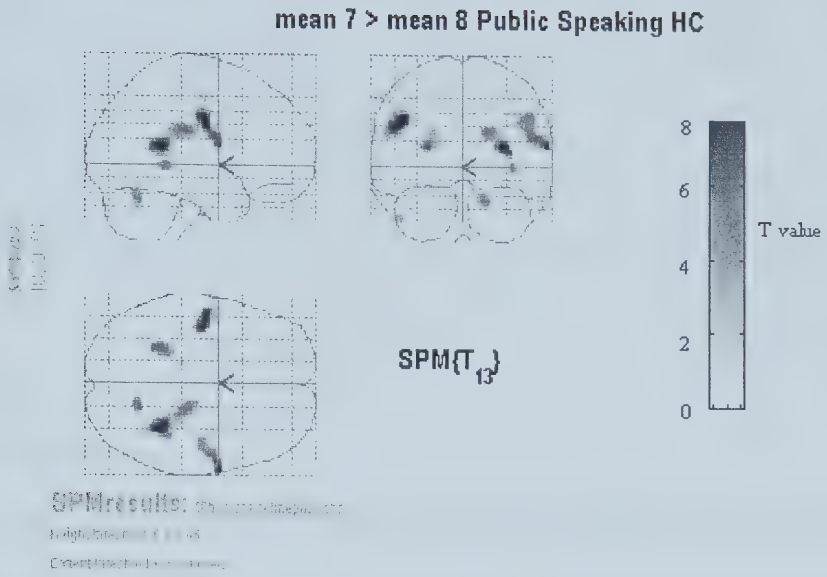

Figure 7-4
Areas of increased activation during public speaking versus public speaking baseline condition, in HC subjects. Darker areas of gray represent areas of higher activation and of higher T-value (corresponding to the gray scale).

Brain Regions Activated	Talairach coor. (mm)			Z-Score	p value (cluster unc)
	x	y	z		
R Temporal lobe (sub-gyral)	32	-40	17	4.60	0.000
L Precentral gyrus (BA6)	-44	-8	34	4.59	0.001
R Precentral gyrus (BA6)	63	1	15	4.21	0.002
L Frontal lobe (sub-gyral)	-26	-40	17	3.89	0.022

Table 7-4
fMRI analysis of HC subjects. Areas of increased activation in HC subjects during public speaking versus public speaking baseline condition. Uncorrected p-value set at 0.001.

7.2 Public Speech vs. Private Speech

GSP subjects (n = 13)**:**

Brain activation in phobic patients was not significantly different during public speech than during private speech at the uncorrected cluster p-value level (*see Figure 7-5).*

HC subjects (n = 14)**:**

Comparing public speech to private speech, nonphobic HCs had increased activation during public speech in the right middle and superior temporal gyrus (*see Figure 7-6; Table 7-5*).

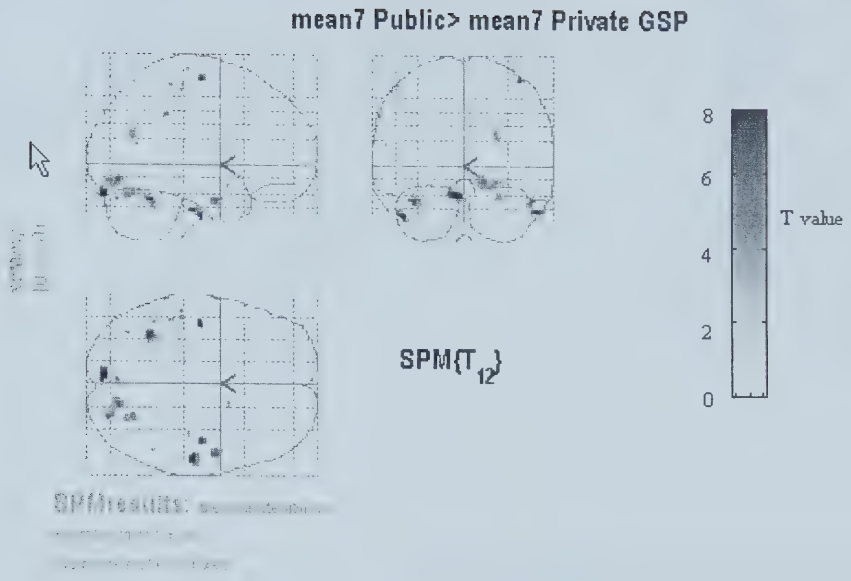

Figure 7-5
Brain areas of increased activation in GSP patients during public speaking versus during private speaking.. Darker areas of gray represent areas of greater activation and of higher T-value (corresponding to the gray scale).

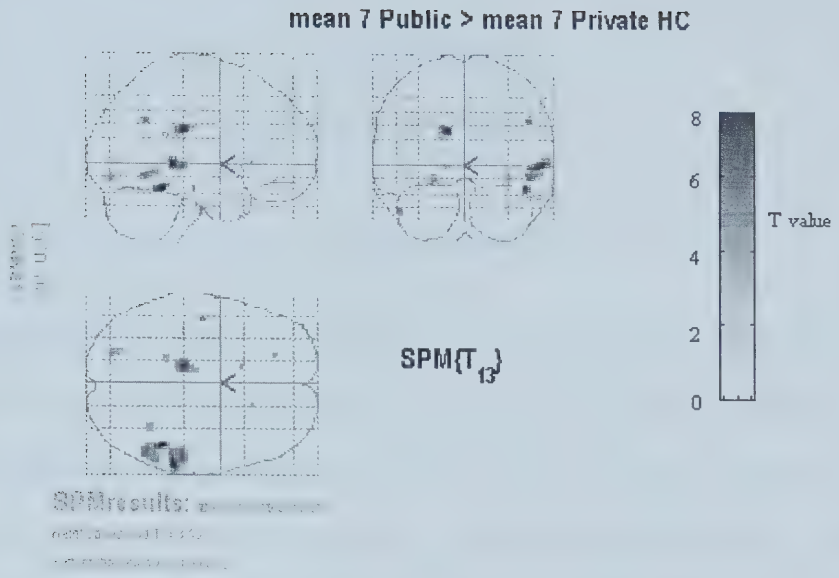

Figure 7-6
Brain areas of increased activation in HC during public speaking versus during private speaking. Darker areas of gray represent areas of greater activation and of higher T-value (corresponding to the gray scale).

Brain Regions Activated	Talairach coor (mm)			Z-Score	p value (cluster unc)
	x	y	z		
R Middle temporal gyrus	59	-35	4	3.91	0.020
R Superior temporal gyrus	55	-27	1	3.65	0.049

Table 7-5
fMRI analysis in HC subjects Areas of increased activation in HC subjects during public speaking versus during private speaking. Uncorrected p-value set at 0.001.

CHAPTER 8

Behavioral Measures

8.1 Visual analogue scale: Present level of anxiety (VAS)

In the GSP group (n=13), a main time effect was observed for the VAS present level of anxiety scale (Huynh-Feldt df = 2.58, $p < 0.01$). There was no significant difference between with the VAS-5min (baseline of private speech) and either VAS pre-speech 1 (private speech) or VAS pre-speech 2 (private speech) ($p = 0.83$; $p = 0.28$). There was a statistically significant difference between VASrest (baseline of public speech) and VAS pre-speech 4 (public speech) ($p < 0.01$). The difference between VASrest and VAS pre-speech 3 approached statistical significance ($p = 0.095$) (see *Figure 8-1; Figure 8-2*). In HC subjects (n = 14), a main time effect was not observed for the VAS scale when the same comparisons were performed (df = 2.72, $p = 0.51$) (*see Figure 8-3; Figure 8-4).*

8.2 Visual analogue scale: Change in level of anxiety (VAS-change)

In GSP subjects (n = 13), a main time effect was observed for the VAS-change scale (Huynh-Feldt df = 2.94, $p < 0.01$). There were no statistically significant differences between the VAS-change [speech 1 and speech 2] or the VAS-change [speech 3 and speech 4] ($p = 0.32$; $p = 0.91$). Mean VAS-change scores were calculated for private (speech 1 and 2) and public (speech 3 and 4) speech. In comparison, the mean VAS-change in anxiety score during private speech was statistically smaller than the mean VAS-change anxiety score during public speech ($p<0.01$) (*see Figure 8-5).*

In the HC group (n = 14), a main time effect was observed for the VAS-change scale (Huyndt-Feldt df = 2.99, $p = 0.037$). Within the tasks, the mean VAS-change speech 1 was not statistically different from the mean VAS-change speech 2 ($p = 0.24$). The mean VAS-change speech 3 (public) was significantly larger than the mean VAS-change speech 4 (public) ($p < 0.01$). Analysis revealed, between the tasks, no significant differences between the VAS-change speech 1 (private) and the VAS-change speech 3 (public) ($p = 0.49$), the VAS-change 1 (private) and VAS-change 4 (public) ($p = 0.13$), or the VAS-change 2 (private) and the VAS-change 4 (public) ($p = 0.44$). The mean score of speech 3 (public) was significantly greater than the VAS-change score of speech 2 (private) ($p = 0.04$) (*see Figure 8-6).*

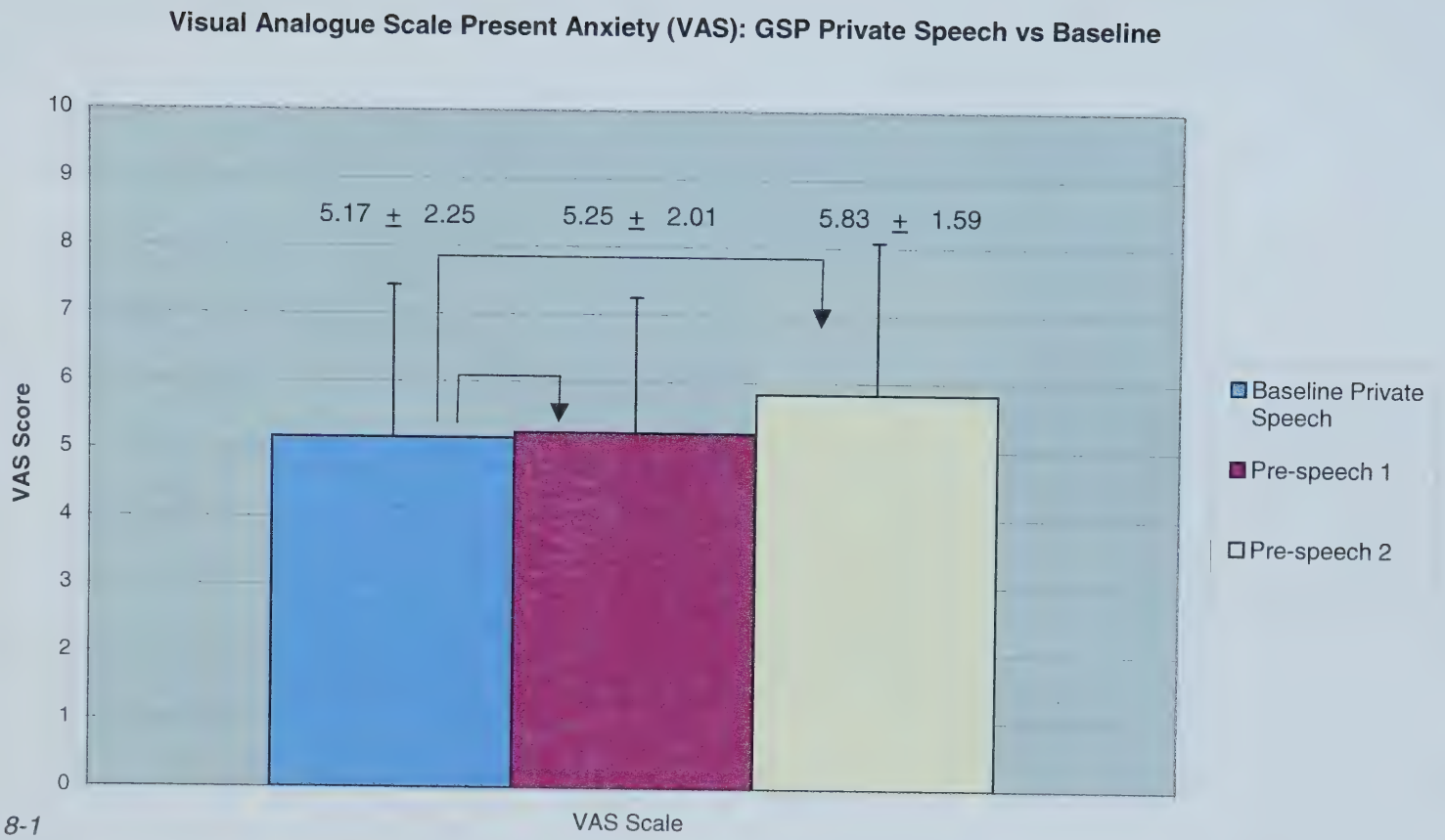

Figure 8-1
Visual analogue scale: present level of anxiety in patients with GSP. A comparison of private baseline subjective report VAS versus private speech VAS (speech 1 and speech 2). Values represent mean ± SD.

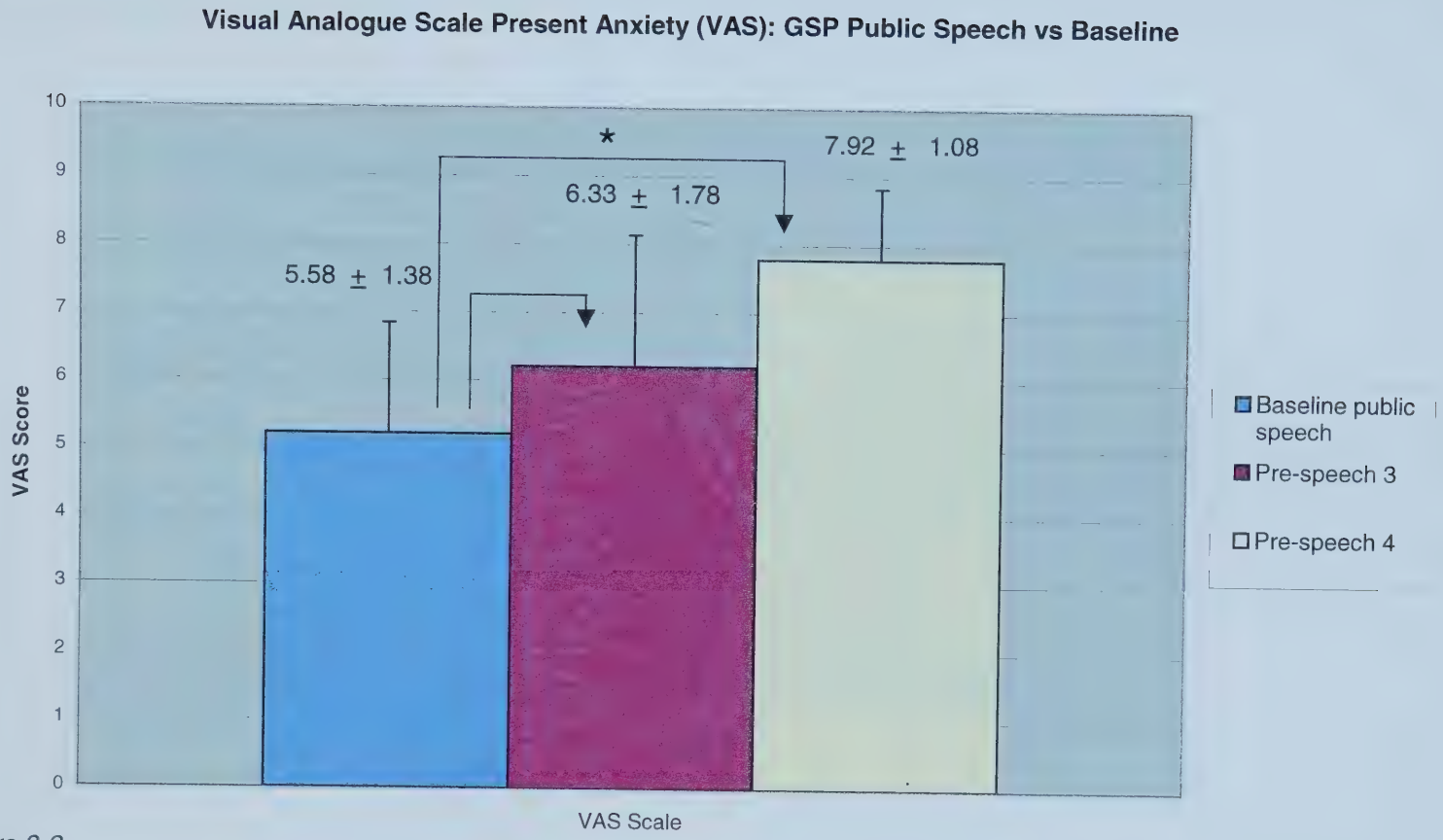

Figure 8-2

*Visual analogue scale: present level of anxiety in patients with GSP. A comparison of publicbaseline subjective report VAS versus public speech VAS (speech 3 and speech 4). Values represent mean ± SD and * represents statistical significance at p<0.05.*

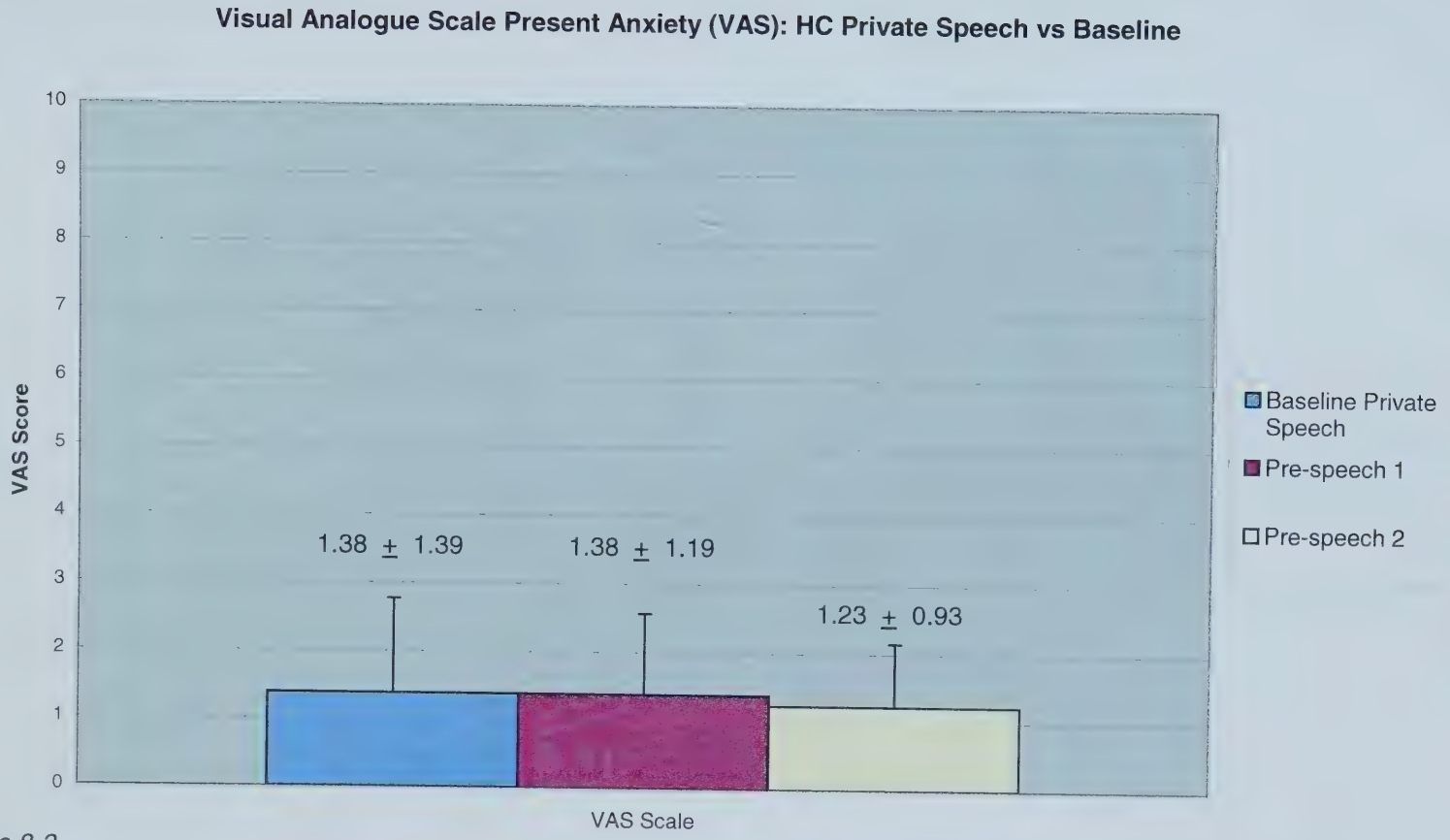

Figure 8-3

Visual analogue scale: present level of anxiety in subjects with HC. A comparison of private baseline subjective report VAS versus private speech VAS (speech 1 and speech 2). Values represent mean ± SD.

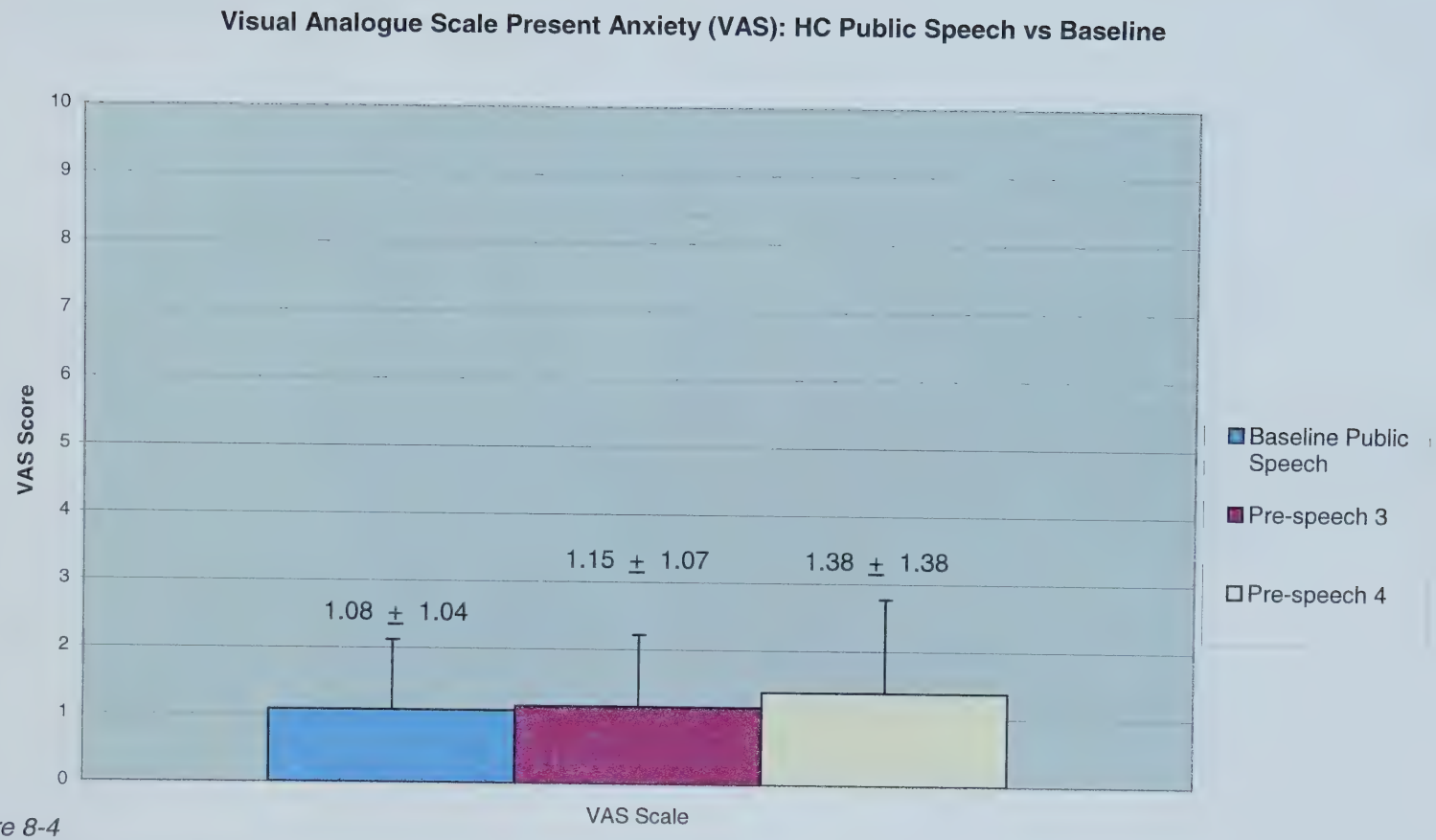

Figure 8-4

Visual analogue scale: present level of anxiety in subjects with HC. A comparison of public baseline subjective report VAS versus public speech VAS (speech 3 and speech 4). Values represent mean ± SD.

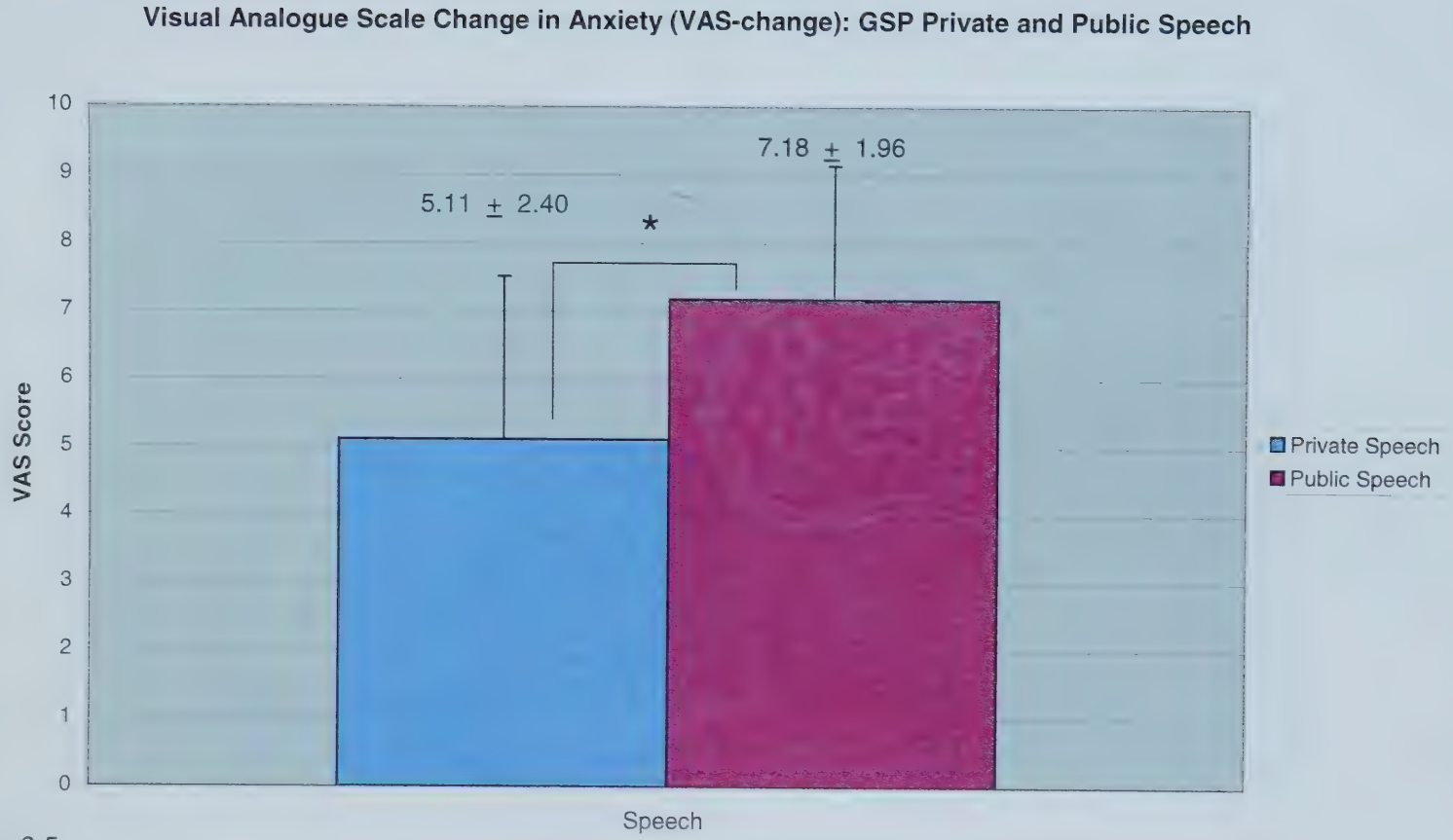

Figure 8-5
*Visual analogue scale: change in level of anxiety in patients GSP. A comparison of change in anxiety (VAS-change) during private speech (speeches 1 and 2) combined) and during public speech (speeches 3 and 4 combined). Values repersent mean ± SD and * represents statistical significance at p<0.05.*

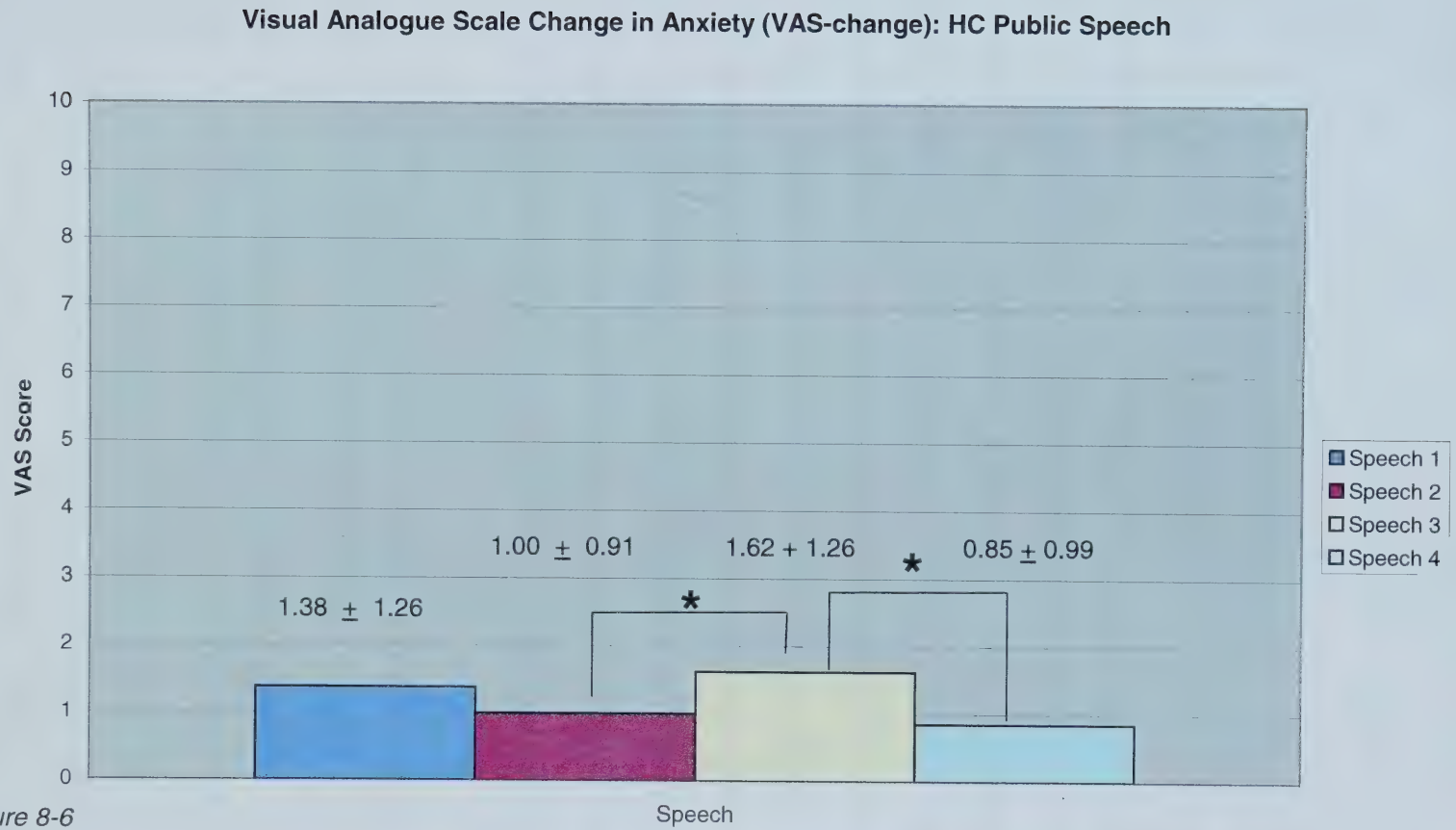

Figure 8-6

*Visual analogue scale: change in level of anxiety in HC. A comparison of change in level of anxiety (VAS-change) in private speaking (speech 1 and speech 2) and public speaking (speech 3 and speech 4). Values represent mean ± SD and * represents statistical significance at $p<0.05$.*

D. DISCUSSION

Discussion

In GSP patients and in HCs, exposure to the private and public speaking tasks compared to the rest conditions prior to speaking (baseline) produced activation bilaterally in the motor cortex (precentral gyri; BA6). In GSP patients the comparison of private and public speech versus baseline private and baseline public, respectively, also produced activation of the cerebellum. In the patient group, the right thalamus, bilateral superior temporal gyri, the right cingulate gyrus and the right fusiform gyrus were activated during public speech when compared to the public baseline condition. In contrast, HC subjects had increased brain activation during public speaking compared to public speaking baseline in the left frontal lobe. A comparison between the speaking tasks, in GSP patients, revealed no significantly different areas of activation. The same comparison in HCs revealed increased activation during public speaking of the right middle temporal and superior temporal gyri when compared to speaking in private.

In both HCs and GSP patients, we expected that a comparison of activation during the speaking tasks and during baseline (where no speaking occurred) would yield significant activation of the motor cortex. Indeed, in both controls and in the GSP population, both the left and right precentral gyri were activated during both speaking tasks. This is consistent with previous literature investigating the neural correlates of the motor components of speech production. Referring to the "motor homunculus" defined by Penfield and Roberts (1959), the middle inferior portion of the precentral gyrus, or

primary motor cortex, is responsible for motor control of the face, lips, and mouth, and jaw (Huang et al., 2001). Activation of the precentral gyri during speaking is also supported by the results of an fMRI study conducted by Huang et al. (2001) that found significantly greater bilateral activation of the middle and inferior regions of the primary motor cortex during overt speech compared to silent speech production.

Initially we hypothesized an alteration in activity in anxiety-relevant brain areas in GSP patients during public speaking. Previous findings in patients with anxiety disorders and in anxiety provocation studies support our results in regard to this hypothesis. Fischer and colleagues (1998) have shown that provoked panic results in decreased rCBF in the cingulate cortex and the temporal cortex. Furthermore, studies of simple phobias have identified several key structures in which activation is altered and perfusion changed during symptom provocation. These include: alterations of rCBF in the hippocampus, prefrontal, orbitofrontal, temporopolar and cingulate cortices; alterations of rCBF in the insula, thalamus and cingulate cortex; and alterations in activation of the inferior frontal cortex, parahippocampal gyri, occipital gyri and fusiform gyri (Johanson et al., 1998; Friedrickson et al., 1997; Rauch et al., 1995). In GSP patients, we have noted altered activation as a result of public speaking in many of these regions, including the right cingulate gyrus, the right fusiform gyrus, the middle and superior temporal gyri, and the right thalamus. Furthermore, the activation we have observed as a result of anxiety provocation is substantiated by studies in patients with PTSD which have demonstrated increased rCBF in the cingulate cortex, cerebellum and

temporal poles following anxiogenic stimuli (Pissiota et al., 2002; Bremner et al., 1999; Shin et al., 1999).

More notably, Tillfors and collegues (2001) have found significantly increased rCBF in the right amygdala during the performance of a public speaking task in SP patients. While we have not observed amygdalar activation, per se, in our study, we have noted a significantly altered activation during public speaking of the temporal gyri, and the right cingulate gyrus, which overlay key limbic structures, including the amygdala. Moreover, the activation of the temporal lobe during the publics speaking task which we observed in GSP patients is supported by Tillfors et al.'s (2001) observations, in SP, of alterated rCBF in the temporal pole during public speaking.

Subjective measures of anxiety reported by the GSP patients reveal a significant increase in the change in anxiety (VAS-change) in GSP patients during the public speaking tasks compared to the private speaking tasks. This result suggests some success in implementing the public speaking fear paradigm in these individuals. Similarly, Tillfors and colleagues (2001) observed significantly increased fear and distress ratings in SP patients during public speaking compared to private speaking.

However, in the GSP group, we observed similar areas and intensity of brain activation during public speech as well as during private speech. We must consider the reasons for a similarity in activation between the theoretically "non anxiety-provoking" (private) and "anxiety-provoking" (public) tasks in the patient population. The first

possible explanation could be that the private speaking task itself was accompanied by a high level of anxiety in the GSP patients. Indeed, the mean change in anxiety (VAS-change) was as high as 5.11 ± 2.40 in GSP patients during the private speaking task. Although statistically significant, this is only slightly smaller than the mean VAS-change score reported during public speaking (7.18 ± 1.96). A two point difference on a ten point scale, with a average standard deviation of 2.18, may not provide a sufficiently large enough difference in increase in blood flow to be detected by fMRI. In particular, because there is already an elevated level of anxiety reported in GSP patients during private speaking, it is likely that brain areas of importance (activated by anxiety) became increasingly activated and perfused during the private speaking task. In this scenario, any further increase in brain activation due to public speaking anxiety would be inconsequential and lacking a significantly increased BOLD response to be captured by fMRI. In the context of this proposed "ceiling effect", we would expect to see few differences in brain areas activated during private speech and during public speech in GSP patients.

Moreover, we did observe a high level of anticipatory anxiety in the GSP population. In the phobic subjects, mean reported baseline anxiety just prior to private speaking (VAS-5min), on the VAS scale, was a 5.17 (on a 10 point scale). This is in contrast to the mean reported baseline anxiety of 1.38 reported by HC subjects at baseline approximately 5 minutes before speaking. Moreover, in GSP patients the baseline anxiety just prior to private speaking is remarkably similar to the mean reported baseline anxiety just prior to public speaking (5.58). From these observations, it appears that

possible explanation could be that the private speaking task itself was accompanied by a high level of anxiety in the GSP patients. Indeed, the mean change in anxiety (VAS-change) was as high as 5.11 ± 2.40 in GSP patients during the private speaking task. Although statistically significant, this is only slightly smaller than the mean VAS-change score reported during public speaking (7.18 ± 1.96). A two point difference on a ten point scale, with a average standard deviation of 2.18, may not provide a sufficiently large enough difference in increase in blood flow to be detected by fMRI. In particular, because there is already an elevated level of anxiety reported in GSP patients during private speaking, it is likely that brain areas of importance (activated by anxiety) became increasingly activated and perfused during the private speaking task. In this scenario, any further increase in brain activation due to public speaking anxiety would be inconsequential and lacking a significantly increased BOLD response to be captured by fMRI. In the context of this proposed "ceiling effect", we would expect to see few differences in brain areas activated during private speech and during public speech in GSP patients.

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expected would occur. In HC patients, subjective reports of change in anxiety during speaking (VAS-change) were higher during speech 1 and during speech 3 (first private and first public speeches) than during speech 2 and during speech 4. HCs also reported a statistically significant increase in anxiety during the first public speech (speech 3) than during the second public (speech 4). As the reported levels of anxiety in the individuals in the HC group are relatively small (ranging from 1.23 to 1.38 on a 10 point scale), this potentially casts a doubt on the clinical relevance of the statistically significant results within the control group. Therefore, we suggest that factors other than anxiety may have played a role in the subjective reports which we collected from the HCs. We hypothesize, rather, that the control group may have been influenced by novelty upon first exposure to each stimulus (private or public conditions). This resulted in higher subjective ratings of the VAS-change during the first exposure to private speech (speech 1) and the first exposure to public speech (speech 3) than the second exposure to private and public speech (speech 2 and speech 4). These results can also be conceived as a habituation to the speaking tasks, with decreased reports during the second private speech (speech 2) and second public speech (speech 4), compared to the first private and public speeches (speech 1 and speech 3).

We are concerned that the subjective reports of anxiety we observed in HCs weaken the validity of assessing the speaking task in this group. The fact that no significant differences in VAS-change were observed between the first private and first public speech (speech 1 and speech 3) or the second private and second public speech (speech 2 and speech 4), suggests that the experience was not significantly altered due to

the condition (private or public) as a whole. Rather the experience in HCs appears to have been altered upon first and second exposure to the stimulus, either private or public. The pattern that we observed in the subjective reports is consistent with the idea that the HC subjects have been influenced by novelty and habituation to the stimulus.

The individuals in our HC group, however, represent a group of people who we consider to be "superhealthy" in terms of their comfort in public speaking situations. In order to maximize our ability to image minute alterations in brain activation, we selected the individuals in the HC group to capture the extreme opposite pole of public speaking discomfort (extremely comfortable). We acknowledge that many differences in the execution and experience of the public speaking paradigm may exist between the non–expert public speakers in the HC population and those with a practiced public speaking background. This may explain the low level of anxiety subjectively reported by individuals in our HC groups.

Additionally, we propose that the subjective reports of increase in anxiety (VAS-change) and pre-speech (anticipatory) anxiety (VAS) in the GSP group can be further dissected to yield some interesting findings. If we consider that the VAS-change measure represents a change in anxiety from before the speech to during the speech and that the VAS scale itself represents the anxiety before speaking, then we can assess the maximum level of anxiety present during the speech (VAS-change + VAS prior to speech). For example, we found no significant differences in the change in anxiety from before to during speaking (VAS-change) for speeches 3 and 4 but observed a significantly higher

level of anticipatory anxiety (VAS) prior to speech 4 than speech 3. Therefore, we suggest that the anxiety experienced DURING speech 4 was greater (7.18 + 7.92 = 15.1) than the anxiety experienced DURING speech 3 (7.18 + 6.33 = 13.5). Based on these findings we hypothesize that GSP patients may have been affected in a manner akin to fear conditioning. In this context, first exposure to the public speaking tasks (speech 3) primed the fear and anxiety response, resulting in a higher anxiety rating prior to the second public speech (VAS speech 4) and a greater level of anxiety during the second public speech (speech 4).

The observation, in HCs, that novelty and habituation may have influenced the experience of the task, as well as the hypothesis that a different level of maximum anxiety was experienced in GSP patients during the two public speeches, highlights the methodological issues associated with repeating the speaking tasks in each condition. That is, the goal of repetition relies on the assumption that within each condition the tasks are identical and responded to identically by the subjects. However, using subjective behavioral measures we have shown that subjects may have experienced the within-condition tasks quite differently, due to novelty and habituation (in HC subjects) and due to varying anticipatory anxiety levels (in GSP patients).

Naturally, the next step to extend our results would be an analysis of between group differences during private and public speaking. Unfortunately, at this time we are limited in our ability to compare an fMRI analysis of the GSP and HC groups. The

complexity of this fMRI paradigm requires unique refinement of standard SPM99 statistical analysis, which is currently underway.

There are several limitations which we acknowledge may be present in this study and should be considered upon analysis of our results. The first limitation is our inability to control the anticipatory anxiety experienced by the GSP patients. While we attempted to control for this problem by allowing subjects the time to accommodate in the scanner for several minutes prior to the start of any speaking, and also by assuring them of their privacy during the private speaking task, we believe that anticipatory anxiety in the GSP patients has influenced our results. We had also initially anticipated that repetition of the speaking tasks may affect the execution and analysis of the speaking tasks. Repetition of the speaking tasks may not adequately represent the occurrence of the same experience twice. In such a complex task it appears that there are many confounding differences which may exist between the repetitions. In the future, to reduce these differences, the speaking task could be performed using an MRI machine with a stronger magnetic field (e.g. 3 T, 4.7 T). A magnet of higher strength would allow for capture of a larger BOLD signal, and thus may eliminate the need for doubling the power of the task or repetition of the task. However, recalling that a higher magnetic field also results in higher magnetic susceptibility to imaging artefacts, the use of a stronger magnet would likely be accompanied by other methodological issues.

The use of a real speaking task in our paradigm, which we believe to be representative of an actual social phobia fear, may also be subject to scrutiny and

criticism. Previous neuroimaging anxiety provocation studies have used visual and auditory stimuli tasks to eliminate head movement of the subject over the course of the scan. We believe that we have adequately investigated the possibility of subject movement artefacts interfering with our results and do not believe that this could have adversely affected our findings. In fact, we have assessed the head motion in all patients to be certain that head movement in each plane (translation and rotation) during the time of each imaging block was not larger than that which can be corrected in the SPM99 pre processing steps. Furthermore, in a recent study of overt speech and silent speech, Huang et al. (2001) assessed head motion during speech. They observed no "extreme head movements" and concluded that head movement was independent of the silent or overt speech conditions, and rather was dependent on the subject performing the tasks (Huang et al., 2001). While there remains some debate among researchers in using real speaking tasks during imaging, we have addressed concerns in this regard and are confident that head motion has not significantly altered out results.

In summary, our results demonstrate increased brain activation in the primary motor area, cerebellum, and temporal lobe, measured by fMRI, in GSP patients during both private and public speaking, compared to baseline conditions. This is in contrast to the limited pattern of activation (increased activation of the primary motor cortex primarily) during private and public speaking which was observed in HC subjects. These results suggest that the use of a "real" speaking task highly impacts brain activation in GSP patients compared to HC subjects with a high comfort level in public speaking.

E. CONCLUSION

Conclusion

This study is the first to assess, using fMRI, brain activation during public and private speaking in GSP patients and HC subjects. We have concluded that, in our paradigm, GSP patients experienced significantly increased brain activation in the primary motor cortex, the cerebellum and in temporal lobe gyri during both private and public speaking, compared to baseline conditions. The same extent of activation was not observed in HC subjects.

Our investigation has also resulted in an assessement of novel methodologies (overt speaking tasks) in the field of fMRI. This study has expanded the idea of using a theoretically "real" anxiety-provoking stimulus (public speaking) in the investigation of SP and explored new design and analysis in fMRI, a discipline which continues to evolve and expand rapidly.

It would appear that a public speaking paradigm is difficult to implement in patients with GSP, who are very sensitive to the suggestion of being exposed to a social situation. The anticipation of this situation in these inidividuals significantly impacts their entire experience (both private and public speaking). In the future, refinement of the statistical analysis, we hope, will lead to a greater understanding of differences in brain activation between GSP patients and HC subjects during speaking.

REFERENCES

Agryropoulos, S.V., Bell, C.J., Nutt, D.J. (2001) Brain function in social anxiety disorder. Psychiatr Clin North Am 24: 707-722.

American Psychiatric Association. *Diagnostic and statistical manual of mental disorders, Fourth Edition*. Washington, DC: American Psychiatric Association: 1994

Attwell, D., ladecola, C. (2002) The neural basis of functional brain imaging signals. Trends Neurosci 25: 621-625.

Ballenger, J.C. (2000) Recognizing the patient with social anxiety disorder. Int Clin Psychopharm 15(suppl 1): S1-S5.

Bandettini, P.A. (2001) Selection of the optimal pulse sequence for functional MRI in *Functional MRI: an introduction to methods (P.Jezzard, P. Matthews, S.Smith, eds).* pp. 123-143. Oxford University Press, Oxford, Great Britain.

Bast, T., Zhang, W-N., Feldon, J. (2001) The ventral hippocampus and fear conditioning in rats: different anterograde amnesias of fear after tetrodotoxin inactivation and infusion of the $GABA_A$ agonist muscimol. Exp Brain Res 139: 39-52.

Biederman, J., Hirshfeld-Becker, D., Rosenbaum, J.F., Herot, C., Friedman, D., Snidman, N., Kagan, J., Faraone, S.V. (2001) Further evidence of association between behavioral inhibition and social anxiety in children. Am J Psychiatry 158: 1673-1679.

Berger, A. (2003) Positron emission tomography. BMJ 326: 1449.

Birmaumer, N., Wolfgang G., Diedrich, O., Uwe, K., Erb, M., Lotze, M., Schneider, F., Weiss, U., Flor, H. (1998) fMRI reveals amygdala activation to human faces in social phobics. NeuroReport 9: 1223-1226.

Boone, M.L., McNeil, D.W., Masia, C.L., Turk, C.L., Carter, L.E., Ries, B.J., Lewin, M.R. (1999) Multimodal comparisons of social phobia subtypes and avoidant personality disorder. J Anxiety Disord 13: 271-292.

Botchin, M.B., Kaplan, J.R., Manuck, S.B., Mann, J.J. (1993) Low versus high prolactin responders to fenfluramine challenge: marker of behavioral differences in adult male cynomolgus macaques. Neuropsychopharmacol 9: 93-99.

Bremner, J.D., Narayan, M., Staib, L.H., Southwick, S.M, McGlashan T., Charney, D.S. (1999) Neural correlates of memories of childhood abuse in women with and without posttraumatic stress disorder. Am J Psychiatry 156: 1787- 1795.

Britten, K.H., Shadlen, M.N., Newsome, W.T., Movshon, J.A. (1992) The analysis of visual motion: a comparison of neuronal and psychophysical performance. J Neurosci 12: 4745-4765.

Brunello, N., den Boer, J.A., Judd, L.L., Kasper S., Kelsey, J.E., Lader, M., Lecrubier Y., Lepine J.P., Lydiard R.B., Mendlewicz, J., Montgomery, S.A., Racagni, G., Stein, M.B., Wittchen, H.U. (2000) Social phobia: diagnosis and epidemiology, neurobiology and pharmacology, comorbidity and treatment. J Affect Disord 60: 61-74.

Buxton, R. (1999) *Introduction to functional magnetic resonance imaging: principles and techniques.* pp. 69, 433. Cambridge University Press, New York, USA.

Cosoff, S.J., Hafner, J. (1998) The prevalence of comorbid anxiety in schizophrenia, shizoaffective disorder and bipolar disorder. Aust N Z J Psychiatry 32: 67-72.

Chapman, W.P., Schroeder, H.R., Geyer, G., Brazier, M.A.B., Fager, C., Poppen, J.L., Solomon, H.C., Yakovlev, P.I. (1954) Physiological evidence concerning importance of the amygdaloid nuclear region in the interation of circulatory function and emotion in man. Science 120: 949-950.

Charney, D.S., Deutch, A. (1996) A functional neuroanatomy of anxiety and fear: implications for the pathophysiology and treatment of anxiety disorder. Crit Rev Neurobiol 10: 419-446.

Chartier, M.J., Hazen, A.L., Stein, M.B. (1998) Lifetime patterns of social phobia: a retrospective study of the course of social phobia in a nonclinical population. Depress Anxiety 7:113-121.

Chavira, D.A., Stein, M.B. (2002) Phenomenology of social phobia in *Textbook of anxiety disorders* (eds D.J. Stein, E. Hollander) pp 290-292. American Psychiatric Publishing, Washington DC, USA.

Crum, R.M., Pratt, L.A. (2001) Risk of heavy drinking and alcohol use disorders in social phobia: a prospective analysis. Am J Psychiatry 158: 1693-1700.

Davidson, R.J. (2002) Anxiety and affective style: role of prefrontal cortex and amygdala. Biol Psychiatry 51: 68-80.

Davidson, R.J. (2000) Affective style, psychopathology and resilience: brain mechanisms and plasticity. Am Psychol 55: 1196-1214.

Davidson, J.R.T., Hughes, D.L., George, L.K., Blazer, D.G. (1993a) The epidemiology of social phobia: findings from the Duke Epidemiological Catchment Area Study. Psychol Med 23: 709-718.

Davidson, J.R.T., Krishnan, K.R.R., Charles, H.C., Boyko, O., Potts, N.L.S., Ford, S.M., Patterson, L. (1993b) Magnetic resonance spectroscopy in social phobia: preliminary findings. J Clin Psychiatry 54 (suppl 12): 19-25.

Davis, M. (1997) Neurobiology of fear responses: the role of the amygdala. J Neuropsychiatry Clin Neurosci 9: 382-402.

Erwin, B.A., Heimberg, R.G., Juster, H., Mindlin, M. (2002) Comormid anxiety and mood disorders among persons with social anxiety disorder. Behav Res Ther 40: 19-35.

Fischer, H., Andersson, J.L.R., Furmark, T., Fredrikson, M. (1998) Brain correlates of an unexpected panic attack: a human positron emission tomographic study. Neurosci Lett 251: 137-140.

Fanselow, M.S. (2000) Contexual fear, gestalt memories, and the hippocampus. Behav Brain Res 110: 73-81.

Fones, C.S.L., Manfro, G.G., Pollack, M.H. (1998) Social phobia: an update. Harvard Rev Psychiatry 5: 247-259.

Fransson, P., Kruger, G., Merboldt, K-D., Frahm, J. (1998) Temporal characteristics of oxygenation-sensitive MRI responses to visual activation in humans. Magn Res Med 39: 912-919.

Friedrikson, M., Fischer, H., Wik, G. (1997) Cerebral blood flow during anxiety provocation. J Clin Psychiatry 58 (suppl 16): 16-21.

Furmark, T., Tillfors, M., Marteinsdottir, I., Fischer H., Pissiota, A., Langstrom, B., Fresrickson, M. (2002) Common changes in cerebral blood flow in patients with social phobia treated with citalopram or cognitive-behavioral therapy. Arch Gen Psychiatry 59: 425-433.

Fyer, A.J., Mannuzza, S., Chapman, T., Liebowitz, M.R., Klein, D.F. (1993) A direct interview family study of social phobia. Arch Gen Psychiatry 50: 286-293.

Godart, N.T., Flament, M.F., Lecrubier, Y., Jeammet, P. (2000) Anxiety disorders in anorexia nervosa and bulimia nervosa: co-morbidity and chronology of appearance. Eur Psychiatry 15: 38-45.

Gordon, E. (1999) Brain imaging technologies: how, what, when and why? Aust N Z J Psychiatry 33: 187-196.

Gore, J.C. (2003) Principles and practice of functional MRI of the human brain. J Clin Invest 112: 4-9.

Heeger, D.J., Huk, A.C., Geisler, W.S., Albrecht, D.G. (2000) Spikes versus BOLD: what does neuroimaging tell us about neuronal activity? Nat Neurosci 3: 631-633.

Heimberg, R.G., Horner, K.J., Juster, H.R., Safren, S.A., Brown, E.J., Schneier, F.R., Liebowitz, M.R. (1999) Psychometric properties of the Liebowitz Social Anxiety Scale. Psychol Med 29: 199-212.

Heimberg, R.G., Hope, D.A., Dodge, C.S., Becker, R.E. (1990) DSM-III-R subtypes of social phobia: comparison of generalized social phobics and public speaking phobics. J Nerv Ment Dis 178: 172-179.

Heydari, B., LeMelledo, J-M. (2002) Low pregnenolone sulphate concentration in patients with generalized social phobia. Psychol Med 32: 929-933.

Hollander, E., Kwon, J., Weiller, F., Cohen, L., Stein, D.J., DeCaria, C., Liebowitz, M., Simeon, D. (1998) Serotonergic function in social phobia: comparison to normal control and obsessive-compulsive disorder subjects. Psychiatry Res 79: 213-217.

Houseman, A.M., Bowtell, R.W. (1999) Functional magnetic resonance imaging: imaging techniques and contrast mechanisms. Phil Trans R Soc Lond B 354: 1179-1194.

Huang, J., Carr, T.H., Cao, Y. (2001) Comparing cortical activations for silent and overt speech using event-related fMRI. Hum Brain Mapp 15: 39-53.

Janz, C., Speck, O., Hennig, J. (1997) Time-resolved measurements of brain activation after a short visual stimulus: new results on the physiological mechanisms of the cortical response. NMR in Biomed 10: 222-229.

Jezzard, P., Clare, S. (2001) Principles of neuclear magnetic resonance and MRI in *Functional MRI: an introduction to methods (P.Jezzard, P. Matthews, S.Smith, eds.).* pp. 67-92. Oxford University Press, Oxford, Great Britain.

Johanson, A., Gustafson, L., Passant, U., Risberg, J., Smith, G., Warkentin, S., Tucker, D. (1998) Brain function in spider phobia. Psychiatry Res 84: 101-111.

Kandel, E.R. (1991) Cellular mechanisms of learning and the biological basis of individuality in " *Principles of Neural Science"* (3rd edition) (E.R. Kandel, J.H. Schwartz, T.M. Jessell, eds) Appleton and Lange. Connecticut, USA.

Kessler, R.C., Stein, M.B., Berglund, P. (1998) Social phobia subtypes in the National Comorbidity Survey. Am J Psychiatry 155: 613-619.

Kim, J.J., Rison, R.A., Fanselow, M.S. (1993) Effects of amygdala hippocampus and periaqueductal gray lesiosn on short- and long- term contextual fear. Behav Neurosci 107: 1093-1098.

Kim, J.J., Fanselow, M.S. (1992) modality specific retrograde amnesia of fear following hippocampal lesions. Science 256: 675-577.

Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H-A., Murison, R., Moser, E.I., Moser, M-B. (2002) Reduced fear expression after lesions of the ventral hippocampus. PNAS 99: 10825-10830.

Kluver, H., Bucy, P.C. (1939) Preliminary analysis of functions of the temporal lobes in monkeys. J Neuropsychiatry Clin Neurosci 1997 (9): 606-20.

Knutson, B., Wolkowitz, O.M., Cole, S.W., Chan, T., Moore, E.A., Johnson, R.C., Terpstra, J., Turner R.A., Reus, V.I. (1998) Selective alteration of personality and social behavior by serotonergic intervention. Am J Psychiatry 155: 373-379.

Koester, J. (1991) Voltage-gated ion channels and the generation of the action potential in " " *Principles of neural science*" (3rd edition) (E.R. Kandel, J.H. Schwartz, T.M. Jessell, eds) Appleton and Lange. Connecticut, USA.

Kupfermann, I. (1991) Hypothalamus and limbic system: peptidergic neruaons, homeostasis, and emotional behavior in " *Principles of neural science*" (3rd edition) (E.R. Kandel, J.H. Schwartz, T.M. Jessell, eds) Appleton and Lange. Connecticut, USA.

LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., Phelps, E.A. (1998) Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron 20: 937-945.

Laubenberger, J. (1997) Magnetic resonance: a multimodal approach to the brain? J Neural Transm Suppl 50: 3-8.

Levin, A.P., Saoud, J.B., Strauman, T., Gorman, J.M., Fyer, A.J., Crawford, R., Liebowitz, M.R. (1993) Responses of "generalized" and "discrete" social phobics during public speaking. J Anxiety Disord 7: 207-221.

Liebowitz, M.R. (1999) Update on the diagnosis and treatment of social anxiety disorder. J Clin Psychiatry 60 (suppl 18): 22-26.

Logothetis, N. (2002) The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. Phil Trans R Soc Lond B 357: 1003-1037.

Malizia A.L., Wilson, S.J., Bell, C.M., Nutt, D.J., Grasby, P.M. (1997) Neural correlates of anxiety provocation in social phobia. NeuroImage 54: S301.

Mathew, S.J., Coplan, J.D., Gorman, J.M. (2001) Neurobiological mechanisms of social anxiety disorder. Am J Psychiatry 158: 1558-1567.

Mennin, D.S., Fresco, D.M., Heimberg, R.G., Schneier, F.R., Davies, S.O., Liebowitz, M.R. (2002) Screening for social anxiety disorder in the clinical setting: using the Liebowitz Social Anxiety Scale. J Anxiety Disord 16: 661-673.

Mick, M., Telch, M. (1998) Social anxiety and history of behavioral inhibition in young adults. J Anxiety Disord 12: 1-20.

Mikkelsen, E.J., Detlor, J., Cohen, D.J. (1981) School avoidance and social phobia triggered by haloperidol in patients with Tourette's disorder. Am J Psychiatry 138: 1572-1576.

Moutier, C.U., Stein, M.B. (1999) The history, epidemiology, and differential diagnosis of social anxiety disorder. J Clin Psychiatry 60(suppl 9): 4-8.

Morris, J.S., deBonis, M., Dolan, R.J. (2002) Human amygdala responses to fearful eyes. Neuroimage 17: 214-222.

Nolte, J. (1999) *The human brain: An introduction to its functional anatomy 4th edition.* pp. 51- 60, 537-563. Mosby Inc., St.Louis, USA.

Nutt, D.J., Bell, C.J., Malizia, A.L. (1998) Brain mechanisms of social anxiety disorder. J Clin Psychiatry 59 (suppl 17): 4-11.

Papp, L.A., Gorman, J.M., Liebowitz, M.R. Fyer, A.J., Cohen, B., Klein, D.F. (1988) Epinephrine infusions in patients with social phobia. Am J Psychiatry 145: 733-736.

Pauling, L., Coryell, C.D. (1936) The magnetic properties and structure of hemoglobin, oxyhemoglobin, and carbonmomoxyhemoglobin. Proc Natl Acad Sci USA 22: 210-216.

Paquette, V., Levesque, J., Mensour, B., Leroux, J-M., Beaudoin, G., Bourgouin, P., Beauregard, M. (2003) "Change the mind and you change the brain": effects of cognitive-behavioral therapy on the neural correlates of spider phobia. Neuroimage 18: 401-409.

Penfield, W., Roberts, L. (1959) *Speech and brain mechanisms.* Princeton University Press. Princeton, USA.

Phillips, G.C., Jones, G.E., Rieger, E.J., Snell, J.B. (1997) Normative data for the personal report of confidence as a speaker. J Anxiety Disord 11: 215-220.

Phillips, R.G., LeDoux, J.E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106: 274-285.

Pissiota, A., Frans, O., Fernandez, M., von Knorring, L., Fischer, H., Fredrikson, M. (2002) Neurofunctional correlates of posttraumatic stress disorder: a PET symptom provocation study. Eur Arch Psychiatry Clin Neurosci 252: 68-75.

Pitman, R.K., Shin, L.M., Rauch, S.L. (2001) Investigating the pathogenesis of posttraumatic disorder with neuroimaging. J Clin Psychiatry 62 (suppl 17): 47-54.

Quirk, G.J., Gehlert, D.R. (2003) Inhibition of the amygdala: key to pathological states? Ann NY Acad Sci 985: 263-272.

Rajagopalan, P., Krishnan, K.R., Passe, T.J., Macfall, J.R. (1995) Magnetic resonance imaging using deoxyhemoglobin contrast versus positron emission tomography in the assessment of brain function. Prog Neuropsychopharmacol Biol Psychiatry 19: 351-366.

Raleigh, M.J., McGuire, M.T., Brammer, G.L., Pollack, D.B., Yuwiler, A. (1991) Serotonergic mechanisms promote dominance acquisition in adult male vervet monkeys. Brain Res 559: 181-190.

Raleigh, M.J., Brammer, G.L., McGuire, M.T., Yuwiler, A. (1985) Dominant social status facilitates the behavioral effects of serotonergic agonists. Brain Res 348: 274-282.

Rauch, S.L., Savage, C.R., Alpert, N.M., Miguel, E.C., Baer, L., Breiter, H.C., Fischman, A.J., Manzo, P.A., Moretti, C., Jenike, M.A. (1995) A positron emission tomography study of simple phobic symptom provocation. Arch Gen Psychiatry 52: 20-28.

Rees, G., Friston, K., Koch, C. (2000) A direct quantitative relationship between the functional properties of human and macaque V5. Nat Neurosci 3: 716-723.

Regier, D.A., Farmer, M.E., Rae, D.S., Locke, B.Z., Keith, S.J., Judd, L.L., Goodwin, F.K. (1990) Comorbidity of mental disorders with alcohol and other drug abuse. JAMA 264: 2511-2518.

Reich, J., Yates, W. (1988) Family history of psychiatric disorders in social phobia. Comp Psychiatry 29: 72-75.

Safren, S.A., Heimberg, R.G., Horner, K.J., Juster, H.R., Schneier, F.R., Liebowitz, M.R. (1999) Factor structure of social fears: the Liebowitz Social Anxiety Scale. J Anxiety Disord 13: 253-270.

Schneier, F.R., Liebowitz, M.R., Abi-Darham, A., Zea-Ponce, Y., Lin, S-H., Laruelle, M. (2000) Low dopamine D_2 receptor binding potential in social phobia. Am J Psychiatry 157: 457-459.

Schneier, F.R., Johnson, J., Horning, C.D., Liebowitz, M.R., Weissman, M.M. (1992) Comorbidity and morbidity in an epidemiologic sample. Arch Gen Psychiatry 49: 282-288.

Schuckit, M.A., Tipp, J.E., Bucholz, K.K., Nurnberger, J.I. Jr., Hesselbrock, V.M., Crowe, R.R., Kramer, J. (1997) The life-time rates of three major mood disorders and four major anxiety disorders in alcoholics and controls. Addiction 92: 1289-1304.

Sheringham, J., Kumari, V., Sumich, A., Sharma, T. (2002) Neuroimaging in schizophrenia: from theory to practice. Hosp Med 63: 328-331.

Shin, L.M., McNally, R.J., Kosslyn, S.M., Thompson, W.L., Rauch, S.L., Alpert, N.M., Metzger, L.J., Lasko, N.B., Orr, S.P., Pitman, R.K. (1999) Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: a PET investigation. Am J Psychiatry 156: 575-584.

Shively, C.A. (1998) Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. Biol Psychiatry 44: 882-891.

Siever, L.J., Murphy, D.L., Slater, S., de la Vega, E., Lipper, S. (1984) Plasma prolactin changes following fenfluramine in depressed patients compared to controls: an evaluation of central serotonergic responsivity in depression. Life Sci 34: 1029-1039.

Spitzer, R.L., Williams, J.B.W., Gibbon, M. (1995) *Structured clinical interview for DSM-IV (SCID)*. New York State Psychiatric Institute, Biometrics Research. New York, USA.

Stanley, J.A. (2002) In vivo magnetic resonance spectroscopy and its application to neuropsychiatric disorders. Can J Psychiatry 47: 315-326.

Stein, M.B., Goldin, P.R., Sareen, J., Zorrilla, L.T.E., Brown, G.G. (2002a) Increased amygdala activation to angry and comtemptuous faces in generalized social phobia. Arch Gen Psychiatry 59: 1027-1034.

Stein, M.B., Jang, K.L., Livesley, W.J. (2002b) Heritability of social anxiety-related concerns and personality characteristics: a twin study. J Nerv Ment Dis 190: 219-224.

Stein, M.B., Chartier, M.J., Hazen, A.L., Kozak, M.V., Tancer, M.E., Lander, S., Furer, P., Chubaty, D., Walker, J.R. (1998) A direct-interview family study of generalized social phobia. Am J Psychiatry 155: 90-97.

Stein, M.B., Leslie, W.D. (1996a) Brain single photon-emission computed tomography (SPECT) study of generalized social phobia. Biol Psychiatry 39: 825-828.

Stein, M.B., Waker, J.R., Forde, D.R. (1996b) Public-speaking fears in a community sample. Arch Gen Psychiatry 53: 169-174.

Stein, M.B., Tancer, M.E., Uhde, T.W. (1992) Heart rate and plasma norepinephrine responsivity to orthostatic challenge in anxiety disorders. Comparison of patients with

panic disorder and social phobia and normal control subjects. Arch Gen Psychiatry 49: 311-317.

Sundet, J.M., Skre, I., Okkenhaug, J.J., Tambs, K. (2003) Genetic and environmental causes of the interrelationships between self-reported fears. A study of a non-clinical sample of Norwegian identical twins and their families. Scand J Psychol 44: 97-106.

Tancer, M.E., Mailman, R.B., Stein, M.B., Mason, G.A., Carson, S.W., Golden, R.N. (1994/1995) Neuroendocrine responsivity to monoaminergic system probes in generalized social phobia. Anxiety 1: 216-223.

Tancer, M.E., Stein, M.B., Uhde, T.W. (1993) Growth hormone response to intravenous clonidine in social phobia: comparison to patients with panic disorder and healthy volunteers. Biol Psychiatry 34: 591-595.

Targum, S.D., Marshall, L.E. (1989) Fenfluarmine provocation of anxiety in patietns with major depressive disorder and panic disorder. Psychiatry Res 28: 295-306.

Thomas, K.M., Drevets, W.C., Whalen, P.J., Eccard, C.H., Dahl, R.E., Ryan , N.D., Casey, B.J. (2001) Amygdala response to facial expressions in children and adults. Biol Psychiatry 49: 309-316.

Tiihonen, J., Kuikka, J., Berstrom, K., Lepola, U., Koponen, H., Leinonen, E. (1997) Dopamine reuptake site densities in patients with social phobia. Am J Psychiatry 154: 239-242.

Tillfors, M., Furmark, T., Marteinsdottir, I., Fischer, H., Pissiota, A., Langstrom B., Fredrikson, M. (2001) Cerebral blood flow in subjects with social phobia during stressful speaking tasks: a PET study. Am J Psychiatry 158: 1220-1226.

Tupler, L.A., Davidson, J.R.T., Smith, R.D., Lazeyras, F., Charles, H.C., Krishnan, R.R. (1997) A repeat proton magnetic resonance spectroscopy study in social phobia. Biol Psychiatry 42: 419-424.

Turner, S.M., Beidel, D.C., Townsley, R.M. (1992) Social phobia: a comparison of specific and generalized subtypes and avoidant personality disorder. J Abnorm Psychol 101: 326-331.

Vieta, E., Corbella, B., Martinez-Aran A., Reinares, M., Benanarre, A., Gasto, C. (2001) Bipolar Disord 3: 253-258.

Weinshenker, N.J., Goldenberg, I., Rogers, M.P., Goisman, R.M., Warshaw, M.G., Fierman, E.J., Vasile, R.G., Keller, M.B. (1996/1997) Profile of a large sample of patients with social phobia: comparison between generalized and specific social phobia. Depress Anxiety 4: 209-216.

Weinstock, L.S. (1999) Gender differences in the presentation and management of social anxiety disorder. J Clin Psychiatry 60 (suppl 9): 9-13.

Whalen, P.J., Rauch, S.L., Etcoff, N.L., McInerney, S.C., Lee, M.B., Jenike, M.A. (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. J Neurosci 18: 411-418.

Wik, G., Fredrikson, M., Fischer, H. (1997) Evidence of altered cerebral blood-flow relationships in acute phobia. Int J Neurosci 91: 253-264.

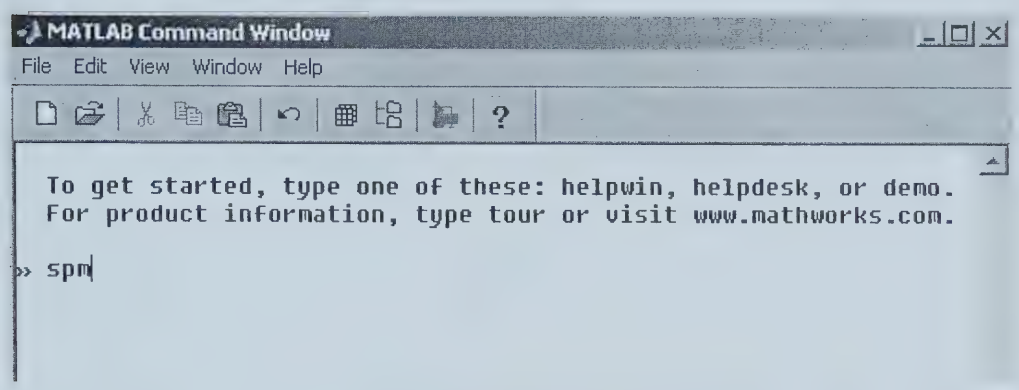
Willson, M.C. (2003) *Implementation of functional magnetic resonance imaging and applications to psychiatric research.* University of Alberta, Dept. Biomedical Engineering M.Sc Thesis, Canada.

Zald, D.H., Pardo, J.V. (1997) Emotion, olfaction, and the human amygdala: amygdala activation during aversive olfactory stimulation. Proc Natl Acad Sci 94: 4119-4124.

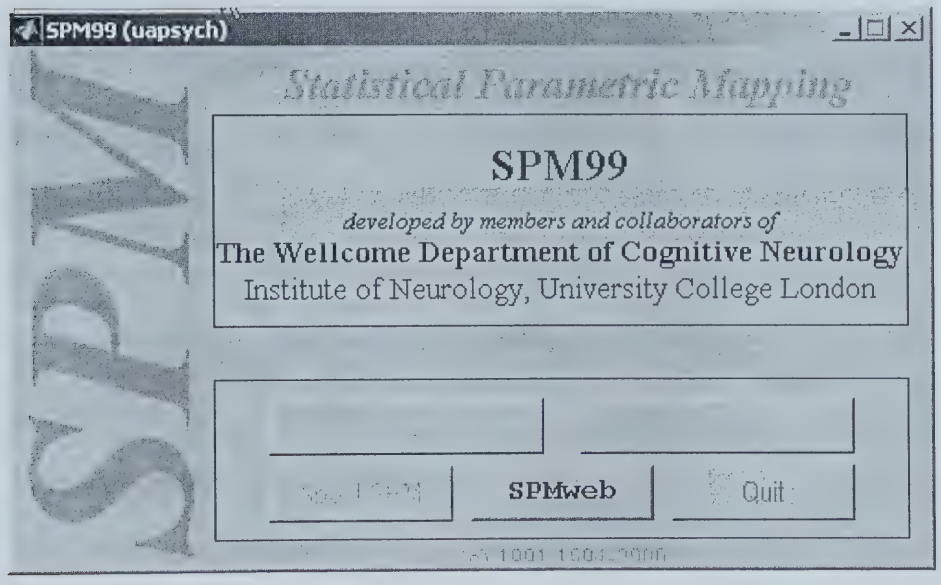
Zola-Morgan, S., Squire, L.R., Alvarez-Royo, P., Clower, R.P. (1991) Independence of memory functions and emotional behavior: separate contributions of the hippocampal formation and the anygdala. Hippocampus 1: 207-220.

APPENDIX I: PROCESSING DATA IN SPM99

SPM99 is a program run off the Matlab software program. To enter SPM99, first open the Matlab program. Type *"spm"* at the Matlab control window and the SPM99 menu will appear.



Select "*fMRI time series*", and the main SPM99 fMRI control pages will be opened.



At any time, the current directory can be specified in the Matlab control window by "cd_x", where x is the directory location you want any new files to be stored in.

```
 ___  ____  __  __
/ __)(  _ \(  \/  )
\__ \ )___/ )    (   Statistical Parametric Mapping
(___/(__)  (_/\/\_)  SPM99 - http://www.fil.ion.ucl.ac.uk/spm


Initialising SPM.........done

SPM present working directory:
        C:\MATLABR11\work
cd

C:\MATLABR11\work

» cd_E://fmrisp
```

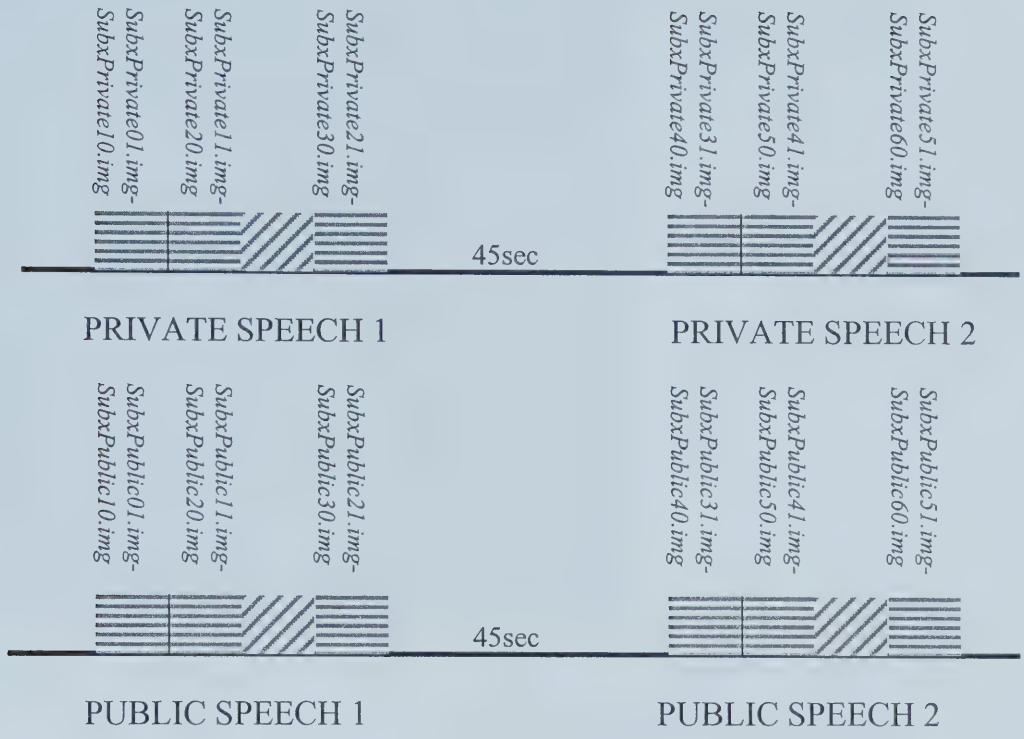
This section will describe, using an example data set, the processes used to carry out the analysis of fMRI data in this study. This fictional data set is composed of one patient with GSP (subject 1) and one HC (subject 2) subject, assuming that each subject underwent the fMRI scanning procedure of the study. For each subject there is a total of 60 images collected during speeches 1 and 2 (private speech session) and 60 images during speeches 3 and 4 (public speech session), each image being compiled from a single volume of images acquired at a single time point in the series.

For each subject (x) the set of images is:

For private speech : *subxprivate01.img, subxprivate02.img, subxprivate03.img...subxprivate60.img*

For public speech: *subxpublic01.img, subxpublic02.img,subxpublic03.img...subxpublic60.img*

Therefore, images obtained for each subject:

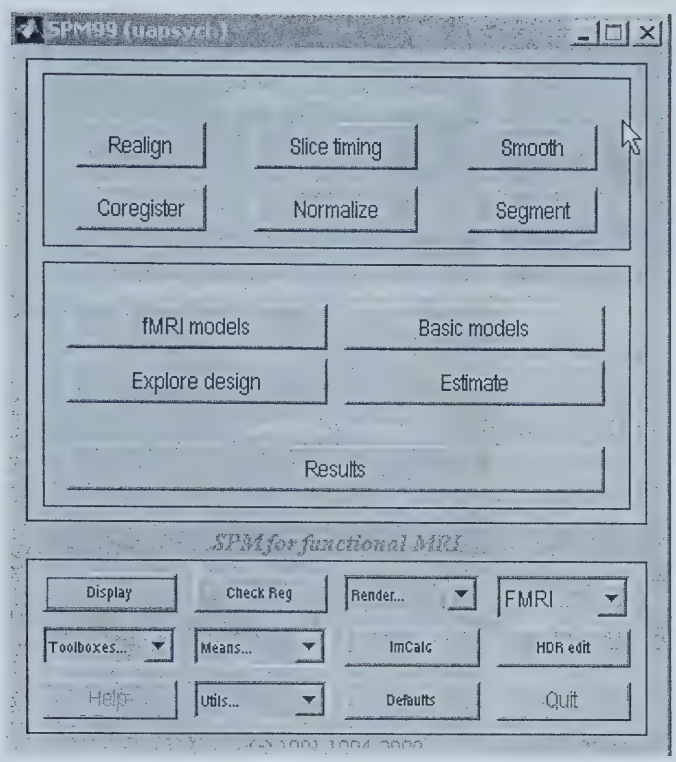


Preprocessing:

Step 1: *Reorientation*

From the SPM99 main menu select:

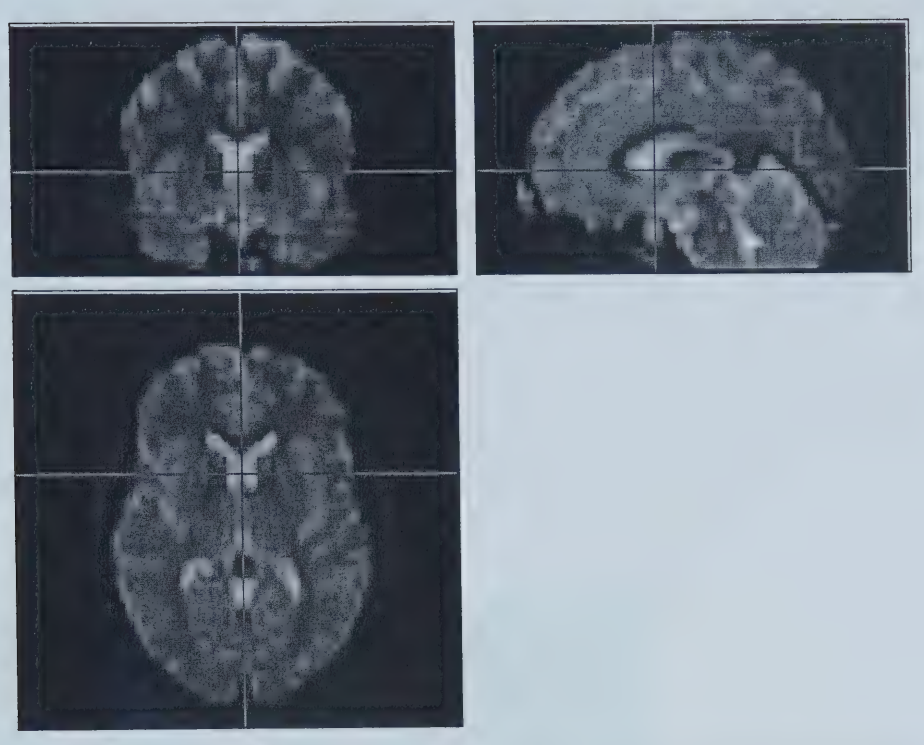
"Display"



Select first image in the set:

subxprivate01.img

This image will open (showing a tranverse, coronal and saggital slice of the image). Crosshairs on this image must line up to the SPM99 template. In the box to specify crosshair position, all images must be flipped along the x and y plane (x = resize –1; y = resize -1) to fit this template. Insert values for the different planes of translation and rotation until the crosshairs on the image are aligned with the template image (roughly equals crosshairs aligning along the anterior comissure).



Once this image lines up in all planes to the template image select:

"Reorient Images"

When prompted to select the images to reorient to the values of translation and rotation of THIS image select:

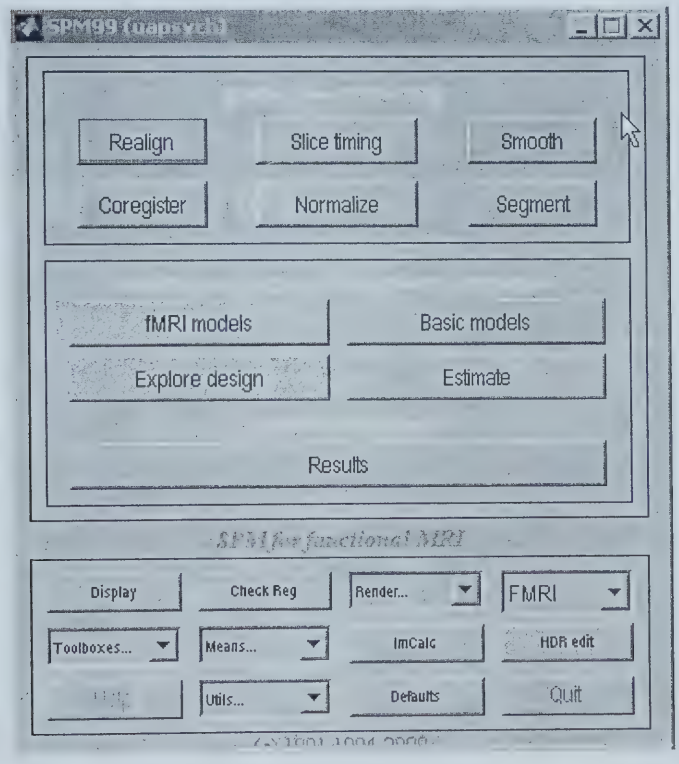
"*1-60subxprivate.img*" (selects all the images 1-60 of private speech)

For each patient (60 private images and 60 public speech images) this reorientation must be done.

Step 2: *Realignment*

From the SPM99 main menu select:

"*Realign*"



Program prompts:

Number of subjects: "**2**"

Number of session per subject: **"12"**

Program prompts selection of images for sub*x*session*x*:

Select each of the 10 images for Subxsession x (pressing "done" after each session or group of 10 images is selected and moving onto the next when prompted):

Session 1: *subxprivate01-10.img*

Session 2: *subxprivate11-20.img*

Session 3: *subxprivate21-30.img*

Session 4: *subxprivate31-40.img*

Session 5: *subxprivate41-50.img*

Session 6: *subxprivate51-60.img*

Session 7: *subxpublic01-10.img*

Session 8: *subxpublic11-20.img*

Session 9: *subxpublic21-30.img*

Session 10: *subxpublic31-40.img*

Session 11: *subxpublic41-50.img*

Session 12: *subxpublic51-60.img*

The selection of these images will be repeated for each subject.

Which option: **"corregister and reslice"**

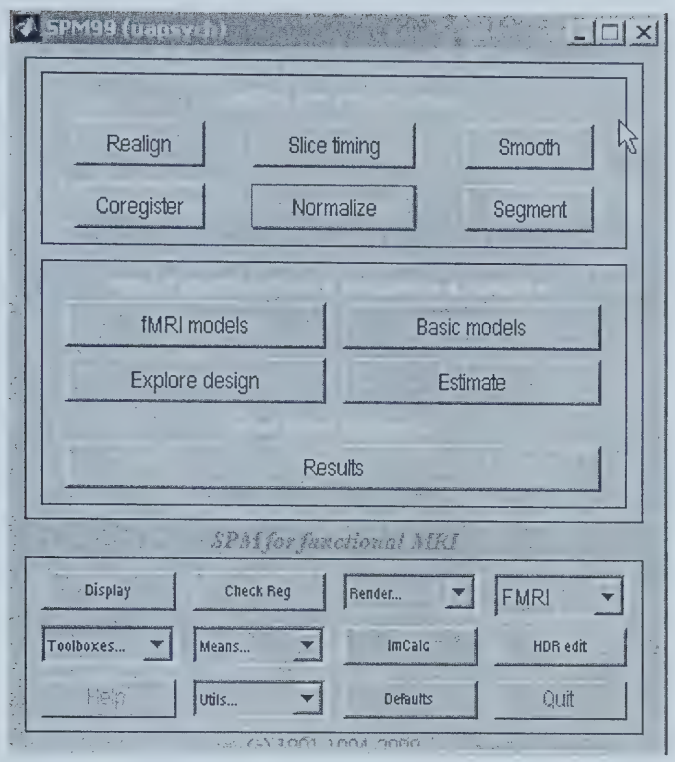
Reslice interpolation method: **"trilinear interpolation"**

Create what: **"mean image only"**

Step 3: *Normalization*

From the SPM99 main menu:

"*Normalize*"



Program prompts:

Which option: **"Determine parameters and write normalized"**

Number of subjects: **"4"** (2 subjects; private and public sessions each)

Image to determine parameters: (select the first image in the private data set)

"subxprivate01.img" – "done"

Select images to write normalize: (select all the images in the private speaking session)

"$_{1\text{-}60}$subxprivate.img" – "done"

Repeat this selection for the public data set and for the second subject, when prompted.

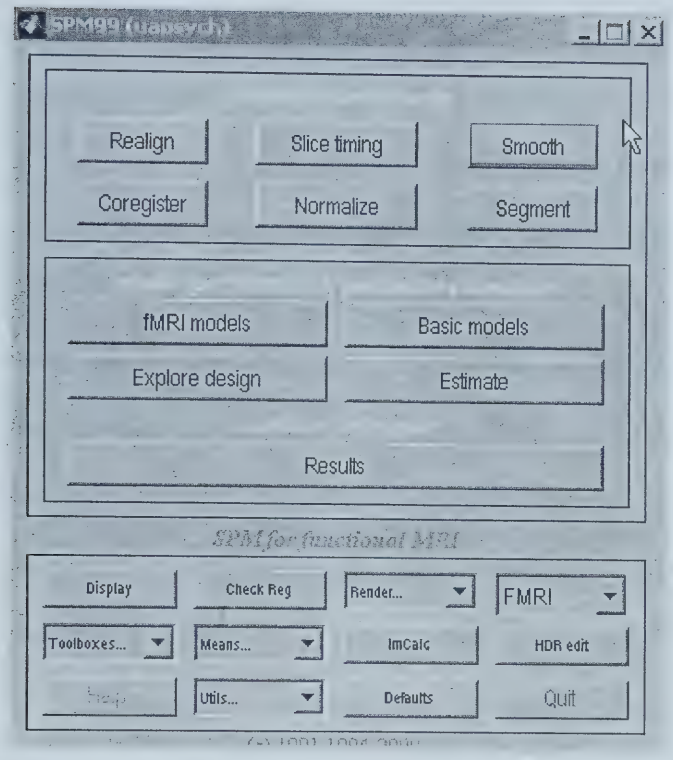
Template image: **"EPI.img"**

Interpolation Method: **"Bilinear interpolation"**

Step 4: *Smoothing*

From the SPM99 main menu select:

"Smooth"



Program promts:

Smoothing (FWHM in mm): **"8"**

Select images: select all the normalized images for all subjects for private and public sessions. **E.g. nsubxprivate01.img…nsubxprivate60.img…**

nsubxpublic01.img…nsubxpublic60.img…

Once all 120 images for all subjects have been selected, chose **"Done"**

Statistical Analysis:

Step 1: *Create mean images*

* Reject first three images for each imaging block*

As mean images are created for each patient for each imaging block, private and public, they must be renamed. In this study the mean images have been renamed as follows:

Private speech 1 baseline before speaking = *meansubxprivate1.img*

Private speech 1 during speaking = *meansubxprivate2.img*

Private speech 1 post-speaking = *meansubxprivate3.img*

Private speech 2 baseline before speaking = *meansubxprivate4.img*

Private speech 2 during speaking = *meansubxprivate5.img*

Private speech 2 post-speaking = *meansubxprivate6.img*

Public speech 1 baseline before speaking = *meansubxpublic1.img*

Public speech 1 (speech 3) during speaking = *meansubxpublic2.img*

Public speech 1 post-speaking = *meansubxpublic3.img*

Public speech 2 (speech 4) baseline before speaking = *meansubxpublic4.img*

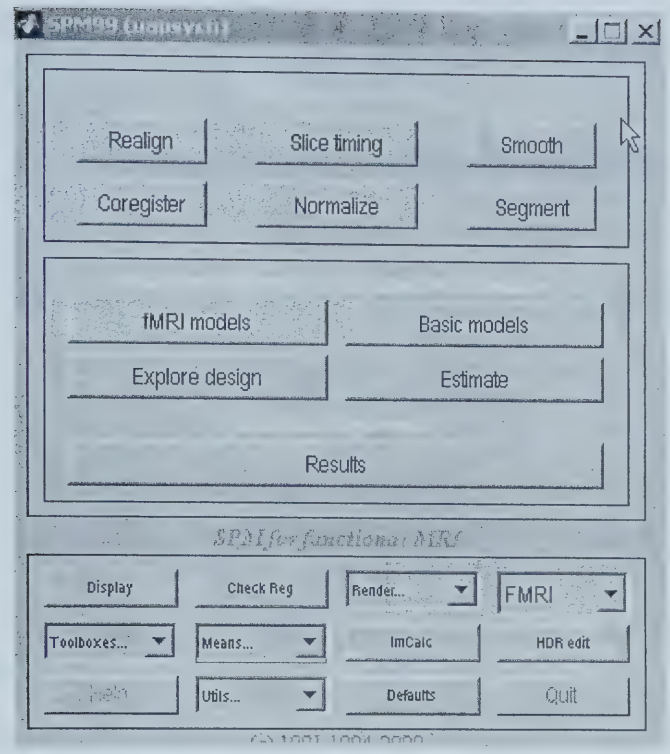
Public speech 2 during speaking = *meansubxpublic5.img*

Public speech 2 post-speaking = *meansubxpublic5.img*

To create means:

From the SPM99 main menu select:

"Means" (from the pull down menu "mean")



Program prompts a selection of the images. For this step the images which have been smoothed and normalized will be used.

Select images: "**snsubxprivate3-10.img**" (to create meansubxprivate1.img)

"**snsubxprivate13-20.img**" (to create meansubxprivate2.img)

"**snsubxprivate23-30.img**" (to create meansubxprivate3.img)

"**snsubxprivate 33-40.img**" (to create meansubxprivate4.img)

"**snsubxprivate 43-50.img**" (to create meansubxprivate5.img)

"**snsubxprivate 53-60.img**" (to create meansubxprivate6.img)

After the images to create one mean image are selected: **"Done"**

Every time a mean image is created it is placed in the current directory and must be renamed before creating other means. For each mean, the current directory will show

"*mean.img*" and "*mean.hdr*" files. Both files are renamed for each mean image as above, preserving their hdr or img endings.

E.g. **meansubxprivate1.img; meanusbxprivate1.hdr**

For each subject 12 mean images are created, 6 for private speech session (meansubxprivate1-6) and 6 for public speech session (meansubxpublic1-6).

Step 2: *Create combined mean images*

For each patient 4 combined mean images are created to form a collective mean image for baseline in the private speech session, speaking during the private speech session, baseline in the public speech session, and speaking during the private speech session.

From the SPM99 main menu select:

"Means" (from pull down menu "mean")

Program prompts a selection of the images:

*Select images: "***meansubxprivate2.img and meansubxprivate5.img"** (To create combined mean during speaking in private session)

OR

"**meansubxpriavte1.img and meansubxprivate4.img**" (To create combined mean baseline prior to speaking)

After selection of the images to form one mean, select "**Done**". Rename these means as in the individual means and according to the following:

"*mean.img*" and "*mean.hdr*" files. Both files are renamed for each mean image as above, preserving their hdr or img endings.

E.g. **meansubxprivate1.img; meanusbxprivate1.hdr**

For each subject 12 mean images are created, 6 for private speech session (meansubxprivate1-6) and 6 for public speech session (meansubxpublic1-6).

Step 2: *Create combined mean images*

For each patient 4 combined mean images are created to form a collective mean image for baseline in the private speech session, speaking during the private speech session, baseline in the public speech session, and speaking during the private speech session.

From the SPM99 main menu select:

"Means" (from pull down menu "mean")

Program prompts a selection of the images:

*Select images: "***meansubxprivate2.img and meansubxprivate5.img"** (To create combined mean during speaking in private session)

OR

"**meansubxpriavte1.img and meansubxprivate4.img**" (To create combined mean baseline prior to speaking)

After selection of the images to form one mean, select "**Done**". Rename these means as in the individual means and according to the following:

"*mean.img*" and "*mean.hdr*" files. Both files are renamed for each mean image as above, preserving their hdr or img endings.

E.g. **meansubxprivate1.img; meanusbxprivate1.hdr**

For each subject 12 mean images are created, 6 for private speech session (meansubxprivate1-6) and 6 for public speech session (meansubxpublic1-6).

Step 2: *Create combined mean images*

For each patient 4 combined mean images are created to form a collective mean image for baseline in the private speech session, speaking during the private speech session, baseline in the public speech session, and speaking during the private speech session.

From the SPM99 main menu select:

"Means" (from pull down menu "mean")

Program prompts a selection of the images:

*Select images: "***meansubxprivate2.img and meansubxprivate5.img"** (To create combined mean during speaking in private session)

OR

"**meansubxpriavte1.img and meansubxprivate4.img**" (To create combined mean baseline prior to speaking)

After selection of the images to form one mean, select "**Done**". Rename these means as in the individual means and according to the following:

*Explicit masking: "***no"**

*Global Calculation: "***mean voxel value"**

*Estimate: "***now**"

Once complete, the results of this test are saved as a *.mat file (SPM.mat) in the current directory, therefore every time an analysis is performed a new directory must be created and specified, or the old one will be rewritten.

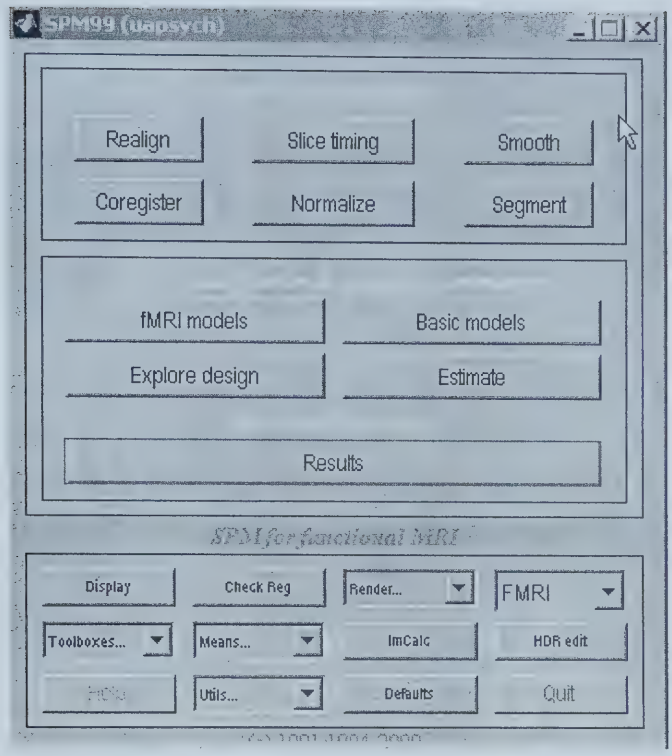
The same process as above can be followed for any comparison to be made by paired t-test, altering for each comparison the number of pairs (number of subjects) and the images selected for the two conditions.

Step 4: *Retrieving the results*

For each paired t-test a *.mat file has been created. To view the results of these tests, the following steps are followed:

From the SPM99 main menu select:

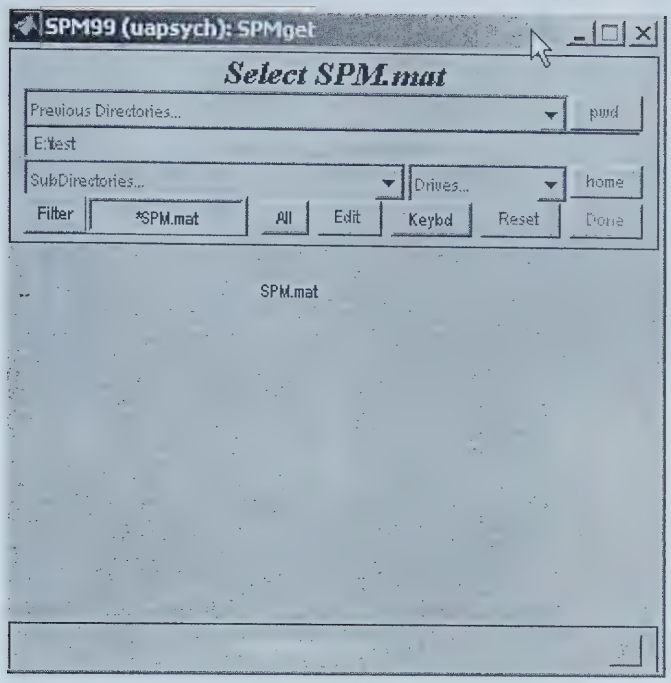
"Results"



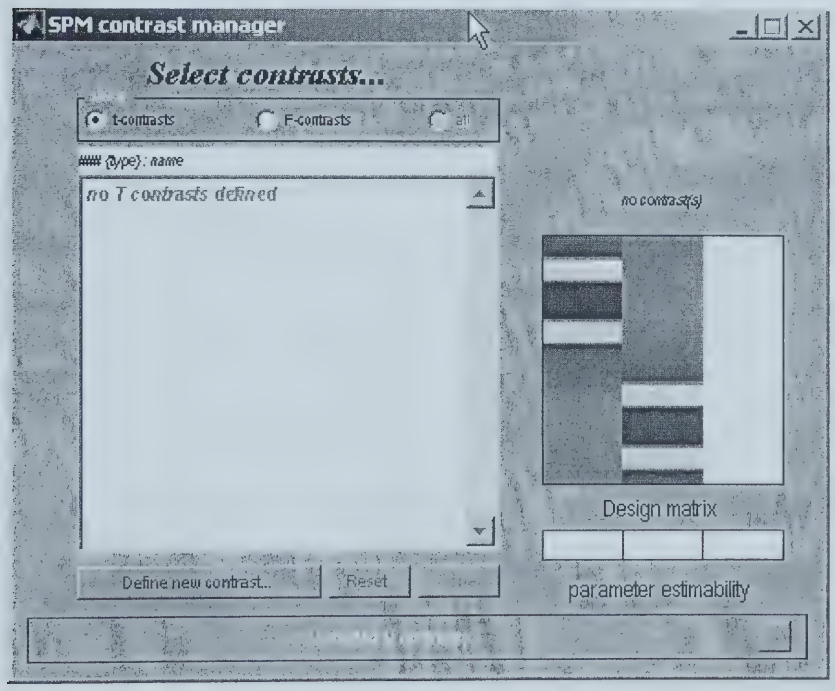
Program will prompt the selection of the *.mat file to be opened

Select: e.g. **"SPM.mat"** (from the directory specified when creating the analysis).

Select: **"done"**

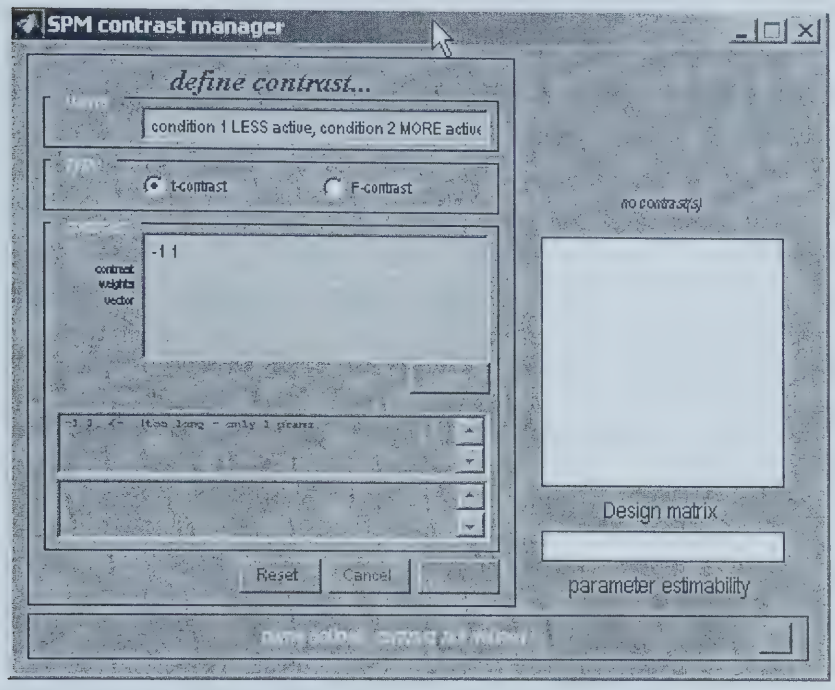


At this point a new t-contrast must be defined for the paired t-test.



Select: **"define new contrast"**

If what is expected to be the LESS active condition as condition 1 (eg. baseline) and the MORE active condition as condition 2 (eg. speaking) then the contrast is defined as: **-1 1**



(This contrast is reversed if the MORE active condition has been selected as condition 1).

At this time the contrast is also renamed, to make it easily identified later on, e.g. "**public speech >baseline public speech GSP**"

*Select: "***o.k."**

*Select: "***done"**

*Mask with other contrasts: "***no"**

*Title for comparison: "***x" (this will correspond with the contrast name)**

Corrected height threshold: **"no"**

Threshold (T or p-value): **"0.001"**

& extent threshold (voxels): **"0"**

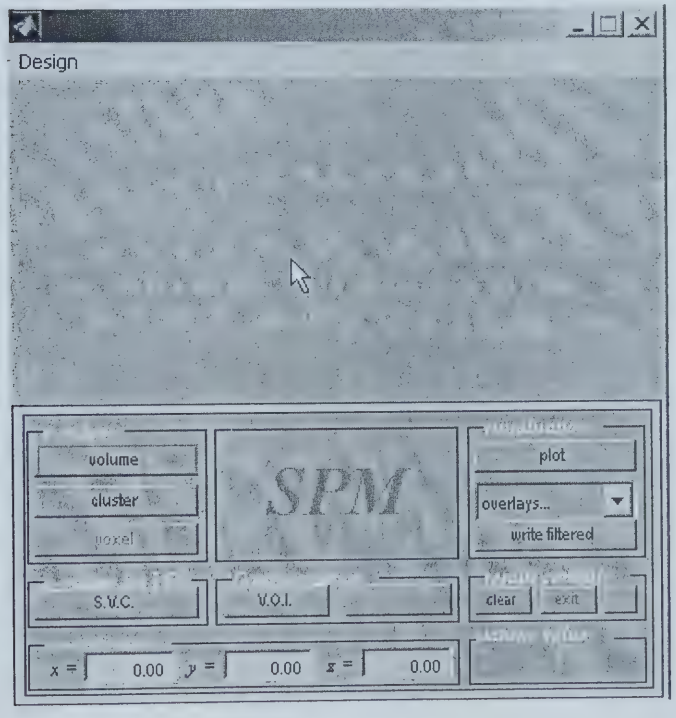
This process will bring up a glass brain, which demonstrates areas where the activation is different between the two conditions.

Step 5: *Displaying the statistical table*

To display the statistical values (threshold values) and coordinates for the voxels in which a difference has been found between conditions 1 and 2.

Once the results of the t-test are displayed using the "retrieve the results" section above:

Select (from the small box at the bottom left): **"volume"**



Statistics: *volume summary p-values corrected for entire volume*

set-level		cluster-level			voxel-level				x,y,z {mm}		
p	c	$p_{corrected}$	k_E	$p_{uncorrected}$	$p_{corrected}$	T	$(Z_=)$	$p_{uncorrected}$			
0.059	21	0.000	3511	0.000	0.000	13.51	5.52	0.000	-42	-18	38
					0.388	9.39	4.83	0.000	-54	-38	26
					0.333	9.34	4.82	0.000	-28	-24	26
		0.000	1153	0.000	0.023	11.78	5.26	0.000	-12	-82	-22
					0.838	33.33	5.29	0.000	26	-54	-24
					0.851	38.58	5.35	0.000	22	-58	-28
		0.000	3017	0.000	0.050	10.53	5.05	0.000	46	-14	42
					0.387	9.43	4.83	0.000	34	-6	24
					0.333	9.36	4.82	0.000	28	-26	46
		0.971	17	0.268	0.275	8.07	4.53	0.000	-4	-46	4
		0.915	24	0.171	0.571	6.94	4.22	0.000	-34	-12	8
		0.010	202	0.000	0.716	6.51	4.09	0.000	20	26	18
					0.738	6.51	4.08	0.000	26	24	24
					1.000	4.58	3.35	0.000	22	18	38
		0.124	104	0.009	0.826	6.15	3.97	0.000	-18	-12	-2
		0.020	179	0.001	0.827	6.15	3.97	0.000	42	-50	-4
					0.962	5.58	3.14	0.000	48	-32	-2
					1.000	4.35	3.25	0.001	34	-38	4
		0.245	80	0.020	0.853	6.08	3.96	0.000	-38	12	-40
		0.013	190	0.001	0.884	5.93	3.89	0.000	82	-18	-2
					0.967	5.46	3.12	0.000	54	-34	-2
		0.826	45	0.066	0.934	5.69	3.81	0.000	44	-10	-24
		1.000	5	0.538	0.948	5.52	3.76	0.000	-38	-82	-2

table shows at most 8 local maxima > 8.0mm apart per cluster

Height threshold: T = 4.02, p = 0.001 (1.000 corrected)
Extent threshold: k = 0 voxels, p = 1.000 (1.000 corrected)
Expected voxels per cluster, <k> = 13.621
Expected number of clusters, <c> = 14.37

Degrees of freedom = [1.0, 11.0]
Smoothness FWHM = 13.5 13.4 12.7 {mm} = 6.7 6.7 6.4 {voxels}
Search volume: S = 1678224 mm^3 = 209778 voxels = 683.8 resels
Voxel size: [2.0, 2.0, 2.0] mm (1 resel = 286.19 voxels)
Page 1

To access a larger version of this table, right click on the table and select "print text table" option. The table is now accessible on the Matlab control window and can be cut and pasted to a word document.

APPENDIX II: T-VALUES, Z-SCORES AND P-VALUES IN SPM99

Based on the analysis of the data, SPM99 generates a t-statistic for each voxel from the t-test. From the t-value obtained SPM99 calculates a z-score for each voxel, which has the same p-value as the t-statistic. While the t-statistic is a reflection of the sample variance, the z-score reflects the population variance. Therefore, conversion to the z-score allows comparison between samples. Furthermore, the t-statistic is calculated based on the degrees of freedom (df) of the sample. The value of the df is indicated on the SPM99 analysis glass brain visual of the results by "SPM{T_{df}}".

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